Fish parasites as bio-indicators of heavy metals in two South African embayments

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Abstract

The Cape Elephant fish (*Callorhinchus capensis*) and two common sand shark species (*Rhinobatos annulatus* and *Rhinobatos blochii*) were caught off False Bay and Saldanha Bay and surveyed for their parasite community in 2013 and 2014. The surveys were used to build species accumulation curves (SAC) and calculate biodiversity indices, particularly, rarefied species richness, Shannon Weiner's diversity index, Simpson's index and Pielou's J index. The biodiversity indices were correlated with the host's biological data and parasite infection data, to determine the parasite community structure and provide insight into the host's community structure. The parasites identified in *C. capensis* (n=19) include a cestode (*Gyrocotyle plana*), two monogeneans (*Callorhynchicotyle callorhynchi* and *Callorhinchicola multitesticulatus*) and an isopod (*Anilocra* sp.). The cestode was the most prevalent at 68.4 % and the monogenean, *C. callorhynchi* was the most abundant (1.68 ±0.78) and had the highest infection intensity (4.00 ±1.45). The SAC and biodiversity measures indicate a uniform parasite community across the host population, suggesting a highly interactive shark community. Conversely, *Rhinobatos annulatus* (n=19) and *R. blochii* (n=17) had very limited parasite infection with two species of nematode found infecting the stomach (*Proleptus obtusus*) and encysted in the kidneys (*Ascaris* sp.) and a copepod species (*Clavelottis* sp.) found infecting the gills. *Proleptus obtusus* was the most prevalent (31.6 % and 29.4%), the most abundant (1 ±0.37 and 3.68 ±2.76) and had the highest mean infection intensity (3.17 ±0.4 and 14 ±1.5). A cestode (*Trilocularia* sp.) was found infecting three specimens of *R. annulatus* from False Bay. The SAC and biodiversity indices combined with the limited parasite infection indicate a non-uniform parasite community across the host population, suggesting an isolationist population. Within the parasite community discovered, a potential biological indicator for heavy metal accumulation was identified to determine the levels of heavy metal pollution within these two anthropogenically impacted bays. *Gyrocotyle plana* and *Proleptus obtusus* were chosen as potential indicators due to their high prevalence and the close relationship they have with their hosts. The results support the use of higher trophic level animals as biological indicators. The results also indicate that *G. plana* is an incredibly good accumulator of certain metals, particularly As (4073.52 ± 5561.54 µg/g), Mn (522.16 ± 578.21 µg/g), Pb (64.87 ± 101.7 µg/g), Ti (1821.42 ± 1348.16 µg/g), and Zn (12439.57 ± 9743.60 µg/g). Unfortunately water and sediment samples were not tested, however, concentrations were compared to baseline values, and the accumulation of these metals are orders of magnitude above the surrounding environment. *Proleptus obtusus* did not significantly accumulate metals from its surrounding environment. These results show that parasites can be used to infer their own and their host's community structure and confirm their usefulness as indicators of pollution in marine ecosystems.

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Chapter 1

General Introduction

1.1: General introduction

1.1.1 Overview

'Food is the factor that plays the biggest parts in their lives, and it forms the connecting link between members of the community' (Elton, 1927). This is the basis for food web theory and has become one of the largest and well-studied fields in ecology. It works off the theory that food is the common currency of communities and distinct patterns within these communities begin emerging once the feeding relationships are understood (Sukhdeo and Hernandez, 2005). As part of these studies, understanding which organism is the top predator is imperative, as it is expected that this organism has major structuring effects down the food web. For example; the lion in the African savannah, or the wolf that has recently been introduced back into the Yellowstone National park, or the great white shark off various coasts around the world. It could be said that humans are the largest predator, as they have the ability to modify ecosystems on a whim. However, by moving into a realm that is still very young in the ecological theatre, challenging this thought becomes possible.

Of all the levels found in the food chain, there is one type of consumer that still feeds off them all. Parasites. Even while reading this, there are a plethora of parasites potentially feeding off various parts of the human body, on a daily basis. This symbiotic relationship can vary from no or little significance to having major pathological effects, resulting in blindness, major morphological changes or even death, just to name a few. From the unassuming common bed bug (*Cimex lectularius*), which thrives in your mattress and feeds on your blood while you sleep, to the more serious *Plasmodium* sp. which are protozoan's that causes Malaria. At some point in our lives the effects of parasites have impacted the world around us, either through the de-worming of our pets to our loved ones contracting tick bite fever or even some horrendous tropical disease. It is through these and other experiences that parasites could be considered major contributors to ecosystem functioning and can have major controlling effects on their hosts and further down the food web.

However, even with the major effects that are witnessed on our own species as a 'tempting' host, the role of parasites in ecosystem functioning has been considered trivial because a cursory examination reveals that their relative biomass is low compared with that of other trophic groups

(Hudson et al., 2006). These parasitic organisms are also small, short-lived and rarely observed in the external environment and during their parasitic phase, are more commonly hidden within their hosts. Therefore, how can it be expected that parasites have the capabilities to structure entire ecosystems and the communities that utilize these systems?

1.1.2 The Negative impact of Parasites

Parasites have an incredibly bad reputation in the animal kingdom, as they utilize virtually every organ and tissue on their host's body as niches to survive (Poulin and Morand, 2000). As much as these hosts try to rid themselves of hitchhikers, the parasites evolve new ways of bettering these defences (Hudson, 2005). Not only have these parasites evolved to cope with and fend off the hosts defences, they have evolved to effect the morphology and appearance of their host, and even change their behaviour, so as to further their own life history goals. Take for example, *Euhaplorchis californiensis*, a digenean of salt-water marshes in Southern California. Its eggs are released in the droppings of shorebirds, the parasite's definitive host. These eggs are ingested by horn snails, which are castrated by the parasite. The parasite then utilizes the snail's reproductive energy to produce cercariae, which are released into the marsh and infect the Killifish (*Fundulus parvipinnis*). The parasite travels up the nerve chord and infects the brain of the Killifish, which causes infected fish to "shimmy, jerk, flash and surface". This alteration in behaviour makes the fish 10–30 times more susceptible to predation by the shorebirds. Once consumed, the parasite infects the digestive tract, completing the parasites life cycle (Lafferty, 2008).

This is just one of the many impacts that parasites impose on their host. Other impacts include imposing energetic demands, affecting morphology and appearance, reducing fecundity and growth, and in the worst case, causing mortality, as outlined in Marcogliese, (2004)

1.1.3 Positive impacts of Parasites

If we observe the impacts of parasites in the individual scale, we can quite easily see how these organisms received their bad reputation. But in medicine they have proved to be rather useful. To delve into the human medical realm, leeches have been used for bloodletting for the last 2500 years and in modern medicine, *Hirudo medicinalis* has been used to reduce swelling and restore blood circulation (Thearle, 1998). However, if we move into the biological world and take a step back to observe the impacts of parasites on a community, or even within an ecosystem, we can begin to

understand the overall importance of these organisms in the structuring of populations (Hudson et al., 2006).

Parasites have the ability to modulate how energy flows through ecosystems, affecting predator-prey interactions and altering food web dynamics and community structure (Poulin, 1999). Poulin, (1999) further summarizes the importance of parasites across all the levels of community ecology. There are three distinct ways in which parasites can affect the structure of free-living hosts. First, different hosts have differing susceptibilities to the same parasitic species. Therefore, the parasite may depress the population of some hosts, more than others, controlling the functional importance of certain free living species. Secondly, through pathological affects, a parasite can control the functional importance of certain hosts in general. And thirdly, the parasite may increase its host's importance in the environment by changing its phenotype. These parasite induced changes cause alterations in host morphology, colouration or behaviour that could increase or decrease the availability of certain resources for other species.

1.1.4 Useful Parasites?

Although parasites have a bad reputation and are considered 'reprehensible citizens' of an ecosystem, they have been shown to be incredibly important in the ecology and evolution of these ecosystems (Poulin, 2007). Because of these important roles they play, they make incredibly useful species to monitor changes that are happening in our ecosystems. The usefulness of parasites in environmental monitoring has long been established with three publications summarizing results and identifying trends within the literature by utilizing quantitative methods (Blanar et al., 2009; Lafferty, 1997; Poulin, 1992). What these studies discovered is that eutrophication and metal contamination were the two types of pollution to illicit a significant response by parasite communities, particularly within the digeneans and monogeneans. Majority of these pollution types had a negative effect on the parasite population by impacting the community richness. According to Mackenzie (1999), there are three reasons why parasites make such excellent indicators of ecosystem health. Firstly, there are more parasitic than free living species that demonstrate an incredible biological diversity as they have had to adapt to a variety of hosts and living environments. Secondly, many parasitic species have complicated life history strategies, often involving highly sensitive, short lived, free living, developmental stages that are incredibly sensitive to environmental change. Finally, there are also parasites that are more resistant than their hosts to environmental change and tend to increase in number when ecosystems become polluted. This has

allowed researches to identify parasites as important indicators of environmental health and several authors have listed criteria to identify parasitic species that would be suitable for monitoring ecosystem change (e.g.: MacKenzie, 1999; Overstreet, 1997; Williams and MacKenzie, 2003). These include:

1. Parasite species and study host should occur commonly.
2. It should be readily identifiable and not easily confused with other similar looking species infecting the subject host.
3. Both the parasite and host species ecology and life cycle should be reasonably well known as well as their geographical distribution
4. It should have a narrow transmission window for infection of subject host
5. If suspected pollution accumulates in sediment, the parasite should have transmission stages in contact with the sea bed.
6. It should be borne in mind that parasitic species living close to their geographical range are likely to be particularly sensitive to environmental change.

1.1.5 Parasite biodiversity

Species richness is a measurement central to the understanding of community and regional diversity (Gotelli and Colwell, 2001). It allows us to place a quantitative measurement on the comparisons between different sites within an ecosystem, and it is these comparisons that form the basis for community and conservation ecology. Studies of parasite diversity can benefit from the application of species richness measurements, as they provide information on the unseen biodiversity that organisms may be hiding (Dove and Cribb, 2006). Dove and Cribb, (2006) recommend utilizing a method that is well established in ecological surveys but has only recently been used in parasitic surveys, i.e., Species Accumulation Curves (SAC's). SAC's are useful in providing an estimate of the total number of species for a given host population and provides a measure of sampling effort. Properties of SAC's are also incredibly informative of community patterns and the structure of parasitic and host diversity. Dove and Cribb, (2006) warn that knowledge of the true distribution of parasite richness over multiple host-derived and spatial scales is far from complete but SACs can improve the understanding of diversity patterns in parasite and host assemblages. One such example of SAC's usefulness in determining where a parasitic community fits on the interactive-isolationist continuum of Holmes and Price, (1986). The continuum provides information on whether a host's infracommunities are high in similarity (interactive) which leads to a predictable community

of parasites, or an infracommunity that is low in similarity (isolationist) which is indicative of a largely unpredictable suite of parasites. This information can then be used to derive the structure of the host population and the types of interactions they are experiencing.

1.1.6 Heavy metal monitoring

Heavy metals are present at low concentrations in our aquatic environments, but an increase in these metals lead to the bioaccumulation of these pollutants in the organs and tissues of the biota (Retief et al., 2009). Majority of heavy metal monitoring has utilized the measurement of the water column and sediment (see research conducted for the South African marine pollution monitoring programme; Cloete and Oliff, 1976; Cloete and Watling, 1981; Gardner et al., 1983). However, there is a growing body of evidence to suggest that by measuring the tissue concentrations of heavy metals in aquatic organisms, we can establish geographical and temporal variations in the bioavailability of these contaminants due to their ability to be concentrated within organism's tissues and organs (Rainbow, 2002). It is also very important in assessing the health of an organism within the environment and in the determination of an organism's fitness for human consumption (Watling and Watling, 1983).

Considering the mounting evidence of parasites as excellent indicators of environmental change, measuring them for heavy metal accumulation has proven to be useful in quantifying environmental pollution (e.g.: Malek et al., 2007). Current literature suggests that parasites are incredibly suited to heavy metal accumulation, concentrating metals to orders of magnitude above their host (Blanar et al., 2009; Lafferty, 2008; Poulin, 1992). Sures, (2003) provides an overview of intestinal helminths and their usefulness as indicators of heavy metal accumulation. He concludes that cestodes, nematodes and acanthocephalans are more consistent and reliable as indicators of heavy metal pollution compared to their host as they are able to concentrate metals to 27 000 times more than surrounding concentrations and 2 700 times more that their host. Sures, (2003) recommends intensifying the research within this field as establishing more sentinel species within an ecosystem is imperative for the monitoring of anthropogenic impacts within these ecosystems.

1.2 Aims and Objectives

This study aims to identify two parasitic biological indicators within the marine environments of two anthropogenically impacted bays in South Africa for the measure of heavy metal accumulation.

The specific aims of this study are:

- Identify the parasite community present in three commercially relevant fish species, Cape Elephant fish (*Callorhinchus capensis*) and two common sandshark species (*Rhinobatos annulatus* and *R. blochii*) in False Bay and Saldanha Bay, South Africa.
- Calculate individual based species accumulation curves and calculate biodiversity indices in an attempt to understand the parasitic and host community structure.
- Determine the heavy metal concentrations in host and parasite tissues and contrast these data with other studies conducted in South Africa and around the world.
- Establish if *C. capensis*, *R. annulatus* and *R. blochii* and their parasites will provide the best measure for heavy metal accumulation (therefore, establish a new biological indicator species).

1.3 Study localities

Southern Africa is renowned for its rich and varied marine fauna and flora. This incredible biodiversity is attributed to the extreme contrast between the water masses on the east and west coasts of the region (Fig. 1.1). The east coast is characterised by warm tropical and sub-tropical water that flows from the subtropics down the coast till past the Agulhas bank, and is known as the Agulhas current. In contrast, the west coast is characterized by cold temperate water, that drifts northward up the coast. This Benguela current is also composed of upwelled water from the depths off the west coast, caused by the wind swept surface waters being pushed off shore and replaced by the colder, nutrient rich waters below. With the changes in temperature around the coast, come the accompanying changes in marine life. The fauna and flora has been extensively studied with over 12 000 species recognised by science (Branch et al., 1994), and these studies have allowed five distinct coastal and four offshore biogeographical regions to be identified (Sink et al., 2012). These include (see Fig. 1.2):

The cool-temperate Namaqua Bioregion of the west coast and warm-temperate Agulhas Bioregion of the south coast are separated by a broad overlap zone, termed the South-western Cape

Bioregion. The east coast consists of the subtropical Natal Bioregion, merging in the far north of the country into the tropical Delagoa Bioregion, which extends northward into Mozambique. The offshore bioregions consist of, the Atlantic Offshore Bioregion, which extends from Namibia to Cape Agulhas, while the West Indian Offshore Bioregion includes the continental slopes of the south and east coasts, meeting the tropical South-west Indian Offshore Bioregion in northern KwaZulu-Natal. A deep-water Indio-Pacific Offshore Bioregion includes the abyss of the entire east coast.

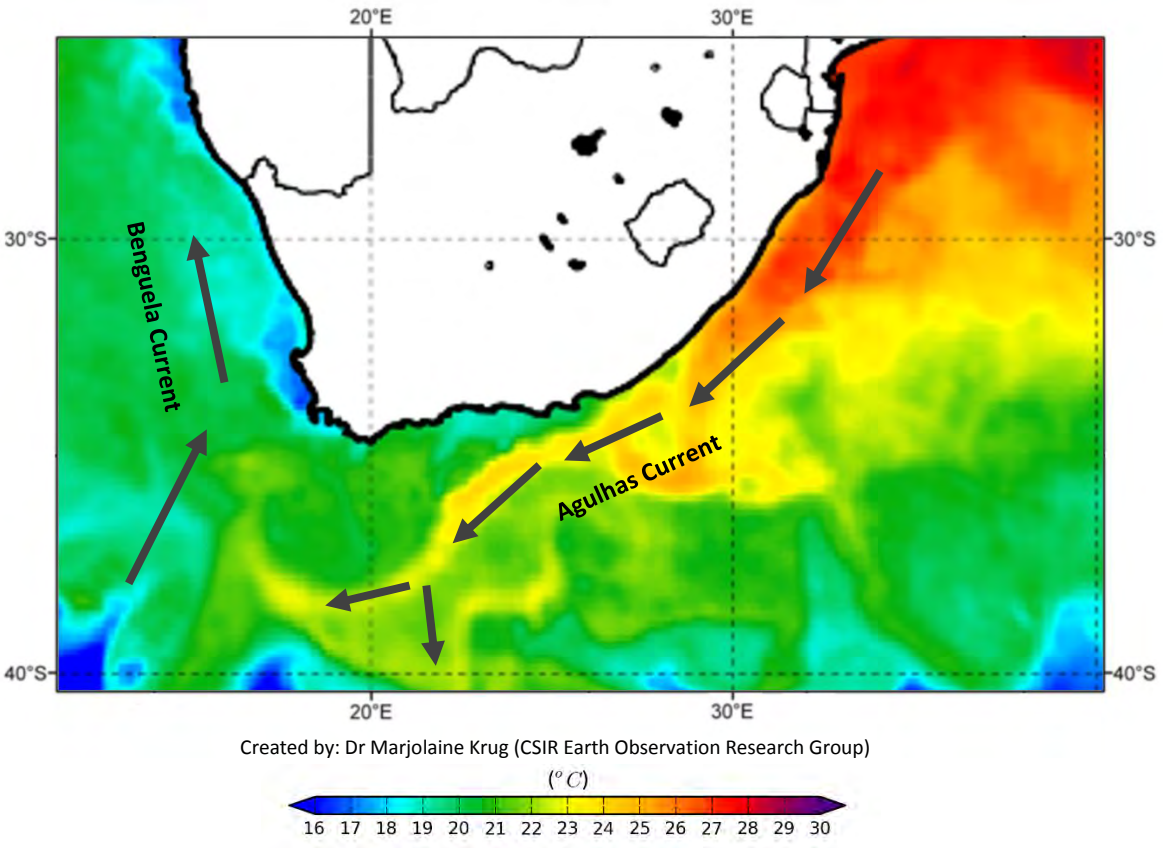

Figure 1.1. Sea surface temperature composite map of Southern Africa, indicating the Agulhas and Benguela ocean current systems, adapted from Reed, (2014) (Global odyssea SST at 0.1 degree resolution derived by the CERSAT). Data from http://www.ifremer.fr/cersat1/exp/productscatalogdetails/) ?id=CER-SST-GLO-1D-010-ODY-MGD).

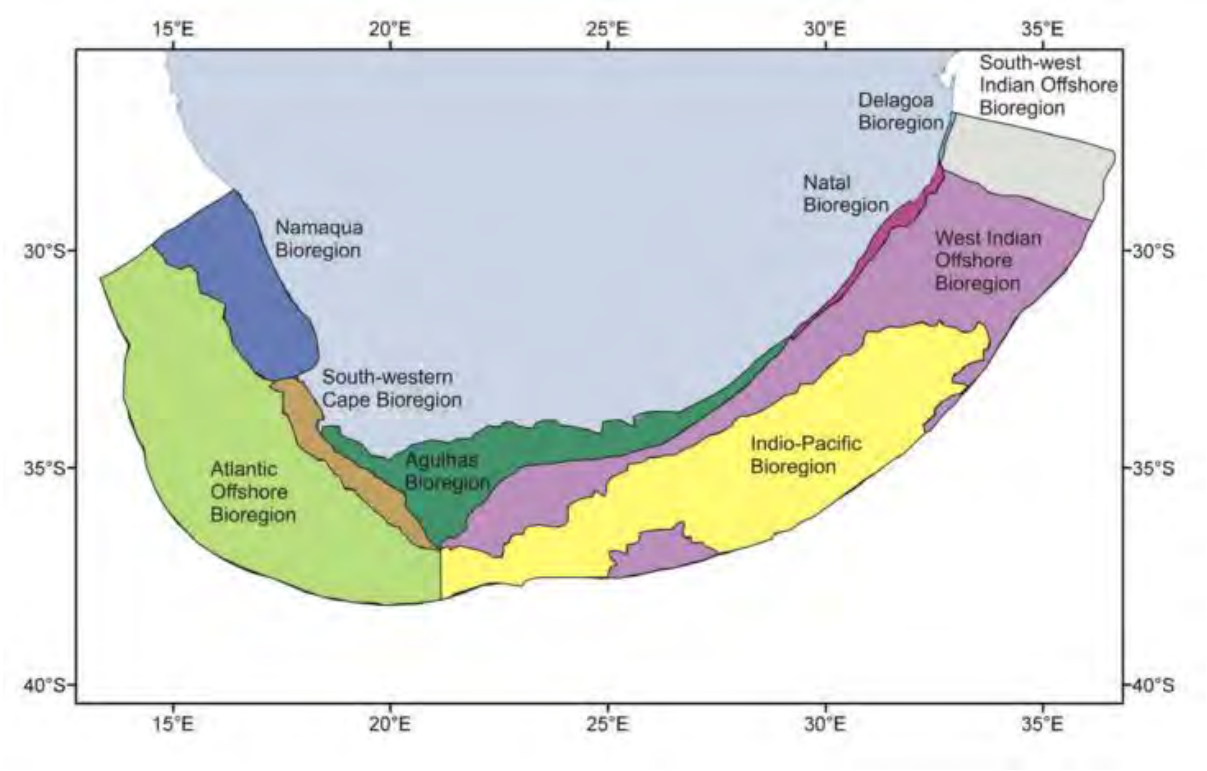

Figure 1.2.: South Africa's nine bioregions, as defined by Lombard, (2004) and adapted from Griffiths et al., (2010).

The locations studied include False Bay and Saldanha Bay (Figure 1.3). Both these bays are situated on the west coast of South Africa and are impacted by commercial and recreational anthropogenic activity.

False Bay is the largest true bay in southern Africa. Situated on the extreme south west of South Africa. It is defined by Cape Hangklip on its eastern shore and the Cape Peninsula on its western shore. Urban development of the coast is intense along some parts of False Bay however; much of the shoreline remains relatively wild and unspoiled. The bulk of the development is residential with little industrial influence. Most pollution is due to non-point sources related to development discharges from storm water drains and effluent pipelines. With the projected increase in population density expected in the city of Cape Town, and that most settlement is occurring on the Cape Flats, this poses an ever increasing threat to the water quality of False Bay (2012/2013 City of Cape Town Annual Report, Reinecke et al., 2012).

Saldanha Bay is approximately 120 km north of Cape Town and is directly linked to the shallow, tidal Langebaan Lagoon. The semi-enclosed bay and lagoon of Saldanha Bay is the only natural harbour of significant size on the west coast of South Africa. It plays host to substantial commercial activities that subject the bay to various pollutant inputs (Atkinson et al., 2006). These include:

- Fish factory effluent from processing plants in the vicinity of the town of Saldanha Bay.
- Debris from the ore jetty which is primarily particulate matter, and consists of iron-oxides which in themselves are not acutely toxic. But, in the near future, the bay will be used as a port for shipping ores of copper, lead, and various other metals. It is a build-up of these elements in the sediments and possibly the biota that is anticipated.
- Oil pollution. The jetty is used for oil offloading procedures.
- Dreading operations to keep the channel clear which has already resulted in the redistribution of faunal communities and loss in diversity.
- Recreational and commercial fishing
- Substantial tourism industry

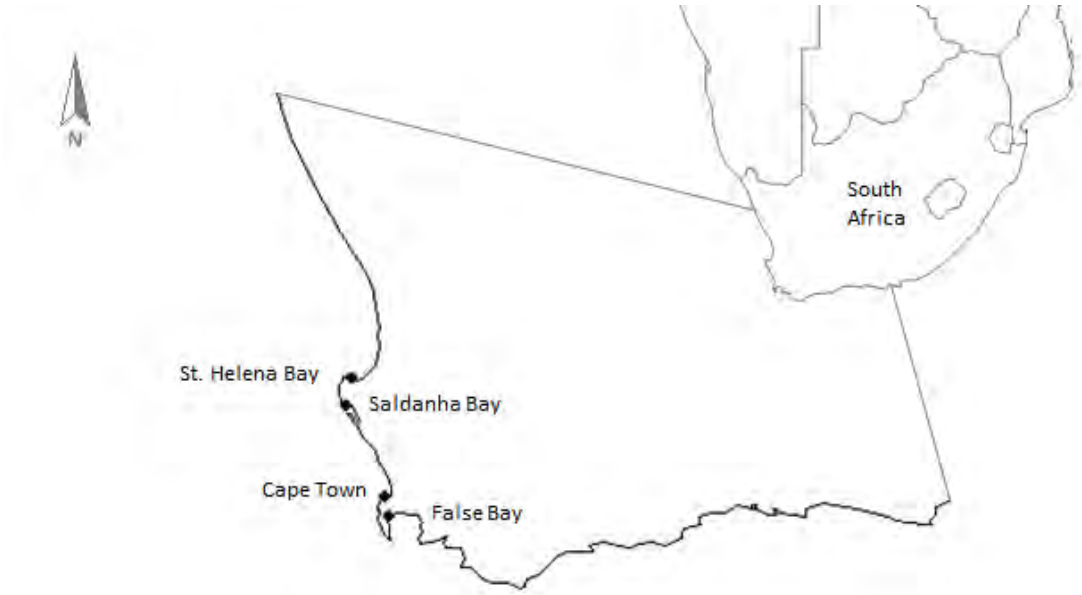

Figure 1.3 Map of Southern Africa indicating study locations (False Bay and Saldanha Bay), with nearby city of Cape Town and town St. Helena Bay for reference

1.4 Outline of thesis

To address the objectives and aims of this Master's thesis, it begins with a preliminary survey of parasites in three species of shark found within the two study sites (Chapter 2 and 3: *Parasites of Callorhinchus capensis*, *Rhinobatos annulatus* and *R. blochii*). All parasites found will be identified, recorded and retained for host-parasite records. Biodiversity measures are calculated and compared to species traits and parasite abundances. Of the parasites found, species of sufficient size will be selected and analysed for heavy metal accumulation along with tissue samples from the respective hosts (Chapter 4: *Use of fish parasites as bio-indicators of heavy metals in South African marine ecosystems*).

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Chapter 2

Parasites of *Callorhinchus capensis* (Duméril, 1865)

2.1 Introduction

Species of the genus *Callorhinchus* are small chondrichthyans of the subclass Holocephali. They are a primitive group of shark which made an appearance early in the fossil record (Carroll, 1988). The genus is restricted to shallow temperate waters in the southern hemisphere, occurring off New Zealand, Chile, Argentina, Australia and southern Africa (Smith and Heemstra, 1986). The three recognised plough-nosed chimeras are the southern African species *Callorhinchus capensis*, the New Zealand/Australian species *C. milii* and the southern American species *C. callorhynchus* (Didier et al., 2012).

Callorhinchus capensis inhabit shallow sandy bottoms in depths up to 374m, with individuals occurring more frequently in shallow water (Freer and Griffiths, 1993a). They have a geographical distribution from KwaZulu-Natal on the east coast (Van der Elst, 1993), to northern Namibia on the west coast (Smith and Heemstra, 1986) (Fig. 2.1). Their distribution may extend into Angolan waters, although no confirmed records currently exist for the area. The shark is a small, smooth, silvery fish which grows to 120 cm in total length (Smith and Heemstra, 1986) and matures at an average of 429mm and 464mm respectively between males and females (Freer and Griffiths, 1993b). They have a characteristic digging proboscis on the front of its snout and the first dorsal fin has a large venomous spine in front of it. There are darker markings on the flanks and head. At maturity, the males have a pair of calcified claspers, paired retractable pre-pelvic graspers and a door-knocker-like projection (tentaculum) on their heads (Fig. 2.1).

These sharks are oviparous, producing dark brown leathery egg cases. The egg case is an elongate oval shape, with a central swelling containing the egg surrounded by a broad laminar frill, which is smooth on one surface and "hairy" on the other. There are no structures to attach it to macrophytes or to the substratum. As the chosen reproductive areas are open sands and muds in sheltered areas, attachment is perhaps either impractical or unnecessary (Freer and Griffiths, 1993a).

Figure 2.1: Drawing of *Callorhinchus capensis* (Smith and Heemstra, 1986) and its distribution along the South African and Namibian coastline.

2.1.1 Commercial importance of *Callorhinchus* spp.

Chimeroids are both targeted in commercial fisheries and taken as either retained bycatch or discarded at sea across the globe. Catches of chimeroids are rarely reported. Historically targeted fisheries have existed for a few species of the genus *Callorhinchus*. *Callorhinchus milli* has been a target species in New Zealand dating back to as early as 1914 (Francis, 1997). From the 1950's to the 1970's there was a steady increase in *C. milli* landings, with a mean of 1075t (Didier et al., 2012). The high demand for *C. milli* was due to export of fillets as well as livers for oil. *Callorhinchus callorhynchus* is caught commercially by trawl fisheries and recreationally. *Callorhinchus capensis* is caught through a directed gillnet and beach seine fishery off the West Coast and False Bay of South Africa. Annual catches are stable at 700 to 900t (<1000t in 2010) with large numbers caught in inshore trawl fisheries (800t in 2010) (Mann, 2013).

Callorhinchus capensis is one of the least-studied commercially exploited fish in South Africa and no information about stock composition currently exists. At present only age, growth, diet and reproductive biology of the species have been documented (Freer and Griffiths, 1993a, 1993b) as well as the macroscopic parasite community documented in Bih Awa, (2012). Chimeroid research is high priority, especially given that many species have restricted distributions and fishing pressures may increase. According to South African marine linefish profiles, this species is "medium" on their research priority and requirements include but are not limited to conducting tests on trace metals and investigating the parasites (Mann, 2013).

2.1.2 Parasites of *Callorhinchus* spp.

Several parasites are known to infect holocephalan species (Table 2.1), with the most prevalent being the gyrocotylideans. Gyrocotylidea is a group of platyhelminthes comprised of only a dozen or so species in three genera: *Gyrocotyle*, *Amphiptyches* and *Gyrocotyloides*. Nearly every holocephalan species so far examined is said to be parasitized by one very prevalent and one rare species of the genus *Gyrocotyle* (Williams et al., 2009). Most authors, who have recognised two sympatric species, have noted that mixed infection never, or very rarely, occur (Simmons and Laurie, 1972). Little is known about their lifecycle as these parasites have not been observed outside their chimeroid host but there are arguments presented by Xylander (1989) to suggest a complex life cycle.

Within South Africa, only recently has there been a concerted effort in studying these unique sharks and their parasites. As early as 1924, Linton described a species of *Gyrocotyle* from the spiral valve of *C. capensis*. It was only after 80 years that *G. plana* was mentioned again by Freer and Griffiths, (1993a), who conducted research on the general biology and fishery for *C. capensis* (Freer and Griffiths, 1993 a, b). The only full parasitic assemblage study on *C. capensis* was conducted by S. Bih Awa, (2012), albeit, this data was never published. Other parasitic studies concerning *C. capensis* are opportunistic taxanomic surveys of species from general trawls off the west coast of South Africa (Beverley-Burton et al., 1993; Manter, 1955).

Apps.webofscience.com was searched for publications containing any combination of the terms *callorhinchus* and *parasit*,* published before June 2014. This initial survey yielded five publications. Of these, only three publications were relevant to *Callorhinchus* and their parasitic species (Amato and Pereira Jr, 1995; Beverley-Burton et al., 1993; Luque and Iannacone, 1991). These search terms were also placed into Google Scholar. Excluding the references found on Web of Science, a further nine relevant papers and unpublished works were recorded (Bih Awa, 2012; Boeger and Kritsky, 1989; Freer and Griffiths, 1993a; Linton, 1924; Manter, 1955, 1954, 1953, 1951; Richardson, 1949). These have been summarized in Table 2.1.

Table 2.1: Parasite records for all Plough-nosed chimeroids (*Callorhinchus* spp.). Table includes location of studies conducted, parasite species, site of infection (SOI), parasite class, and the reference.

Parasite Species	Parasite Class	SOI	Reference
		***Callorhinchus capensis* (South Africa)**	
Gyrocotyle plana	Cestode	Spiral valve	(Linton, 1924); (Freer and Griffiths, 1993a); (Bih Awa, 2012)
Branchellion sp.	Hirudinea	External	(Bih Awa, 2012)
Anilocra capensis	Isopod	External	(Bih Awa, 2012)
Callorhynchicola branchialis	Monogenea	Gills	(Beverley-Burton et al., 1993)
Callorhynchicola multitesticulatus	Monogenea	Gills	(Manter, 1955); (Beverley-Burton et al., 1993); (Bih Awa, 2012)
Callorhynchicotyle callorhynchi	Monogenea	Gills	(Bih Awa, 2012)
		***Callorhinchus callorhynchus* (Uruguay and Argentina)**	
Rugogaster hydrolagi	Aspidogastrea	Rectal Glands	(Amato and Pereira Jr, 1995)
Rugogaster callorhinchi	Aspidogastrea	Rectal Glands	(Amato and Pereira Jr, 1995)
Callorhynchocotyle marplatensis	Monogenea	Gills	(Luque and Iannacone, 1991)
		***Callorhinchus milii* (New Zealand)**	
Macraspis elegans	Aspidogastrea	Gall bladder	(Manter, 1954)
Gyrocotyle rugosa	Cestode	Spiral valve	(Manter, 1953, 1951)
Gyrocotyle urna	Cestode	Spiral valve	(Manter, 1953, 1951)
Branchellion parkeri	Hirudinea	Not Specified	(Richardson, 1949)
Callorhynchicola branchialis	Monogenea	Gills	(Beverley-Burton et al., 1993)
Callorhynchicola multitesticulatus	Monogenea	Gills	(Beverley-Burton et al., 1993)
Callorhynchocotyle amatoi	Monogenea	Gills	(Boeger and Kritsky, 1989)

2.1.3 Aims and Objectives

This chapter aims to identify all metazoan parasites that infect *Callorhinchus capensis* in False Bay, South Africa and build on the knowledge that Freer and Griffiths, (1993a) and Bih Awa (2012) have established. With this knowledge, a parasitic species that fits biological indicator criteria will be identified and used in heavy metal analysis (see Chapter 4). Individual based species accumulation curves will be drawn, biodiversity indices and condition factor will be calculated and compared to parasitic infection to provide a look into the parasitic community structure present in *C. capensis*.

2.2 Methods

2.2.1 Collection and Dissection protocol

Samples of *Callorhinchus capensis* were collected from commercial beach seine net fishermen off the northern coast of False Bay, South Africa between Sunrise Point and Strandfontein in False Bay, intermittently from June 2013 to November 2013 (Table 2.2). A total of 19 sharks were collected, ranging in length from 311 mm to 817 mm. Sharks were removed deceased from the nets and placed in plastic bags. Samples were then transported to the Department of Biological Sciences, University of Cape Town and frozen at -20^{o}C till processing.

Table 2.2: Collection details of samples of *Callorhinchus capensis* caught in False Bay, South Africa.

Year	**Date of capture**	**Sample size (n)**	**Size Range**
2013	June	2	637 - 660
2013	May	9	477 - 817
2013	November	8	311 - 446

Prior to dissection, sharks were thawed individually at room temperature, the sex determined, weighed to the nearest gram (g) and measured for total length and standard length (base of tail) to the nearest millimetre (mm). Measurement was done with the proboscis bent up against the base plate of the measuring board, as recommended by Coakley, (1973). Relative condition factor (CF) was calculated according to the following equation (Froese, 2006; Le Cren, 1951):

$$CF = W / a\, L^{b} \qquad \text{EQN 2.1}$$

Where W = weight (g), L = total length (cm). The exponent *a* and *b* is derived from the length–mass relationship which is described by:

$$W = a\, L^{b} \qquad \text{EQN 2.2}$$

These values were then compared to www.Fishbase.org to see if calculated *b* variables were within range of species norm.

A survey of the parasitic fauna was conducted, as recommended by MacKenzie and Abaunza, (2005). After an external examination for macroparasites, gills were removed and separated into petri dishes, and examined with a dissecting microscope at 10x magnification (Leica EZ4). Sharks were

then eviscerated and organs separated. The alimentary canal was cut open, and the contents examined with a dissecting microscope for parasites. Kidney, liver, muscle, gall bladder, and gonad samples were smeared and examined at 40 x magnification (Leica ICC50, DM750) for microscopic parasites. Any parasites found during these processes were kept in 10% formalin and the count was recorded. Parasites were identified as far as possible with the help of Dr. Cecile Reed (University of Cape Town) as well as using the literature (Beverley-Burton et al., 1993; Bih Awa, 2012; Freer and Griffiths, 1993a; Linton, 1924)

2.2.2 Statistical analysis

Basic infection statistics were collected following the methods of Bush *et al.*, (1997). Prevalence is the number of hosts infected with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species. Mean intensity is the average intensity of a particular species of parasite among the infected members of a particular host species. In other words, it is the total number of parasites of a particular species found in a sample divided by the number of hosts infected with that parasite. Mean abundance is the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined (including both infected and uninfected hosts). It is thus the average abundance of a parasite species among all members of a particular host population. Prevalence, mean intensity and mean abundance was calculated for each parasite species collected.

Species diversity and richness was calculated using rarefied samples across the species community matrix (Gotelli and Colwell, 2001). Species accumulation curves are used to determine the basic information to validate richness comparisons. Sample-based rarefaction curves depend on the spatial distribution of individuals as well as the size and placement of samples (Gotelli and Colwell, 2001). This allows for meaningful standardization and comparison of datasets. It also randomizes data and prevents the impact of the "host effect", differences in host traits that could affect parasite species infection (Dove and Cribb, 2006). Rarefied species richness, Shannon Weiner's diversity index (H) (which takes into account both the number and relative abundance of species), Simpsons index (λ) (the probability that two entities taken at random from the dataset of interest (with replacement) represent the same type) and Pielou's J (J) index (an index of pattern diversity or evenness in the relative abundance of species) were calculated.

Correlations between biological measures and diversity indices were attempted with the use of Spearman's rank order correlation index to confirm statistical significance at 95% ($p<0.05$). The Spearman's rank order correlation or Spearman's rho (r_s) is a non-parametric measure of statistical dependence between two variables. It assesses how well the relationship between two variables can be described using a monotonic function. Chi squared (χ_2) and Mann Whitney U statistical analyses were used to test for the significant affect sex may have on the prevalence and abundance of parasitic infection, respectively. To test for normality, frequency distributions, Q-Q plots and a Shapiro-Wilk tests were used.

All analyses were conducted in either Microsoft Excel (2013) or R 2.15.1 (R Core Team, 2012), with the use of R packages Vegan (Oksanen et al., 2012) for rarefaction and calculation of biodiversity indices

2.3 Results

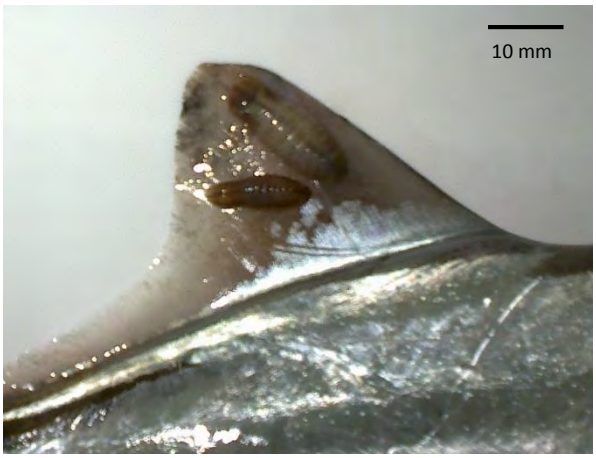
2.3.1 Summary statistics

Four parasitic species were found infecting a total of 19 specimens of *Callorhinchus capensis*. A cestode (*Gyrocotyle plana*), two monogeneans (*Callorhynchicotyle callorhynchi* and *Callorhinchicola multitesticulatus*) and an isopod (*Anilocra* sp.). *Gyrocotyle plana* was the most prevalent at 68 % with the monogenean *C. callorhynchi* the most abundant (1.68 ±0.78) and had the highest average infection intensity (4.00) as well as the greatest variation in infection (Standard error: ±1.45) (Table 2.3; Figure 2.2).

Gyrocotyle plana

Callorhynchicotyle callorhynchi (40X)

Callorhinchicola multitesticulatus (40X)



Anilocra sp.

Figure 2.2: Metazoan parasites found infecting *Callorhinchus capensis* caught in False Bay, South Africa in 2013.

Table 2.3: Summary statistics for parasites found infecting *Callorhinchus capensis* (n=19) caught in False Bay, South Africa during 2013. (SOI = site of infection)

Parasite Class	Parasite Species	SOI	Prevalence (%)	Parasite Abundance (± SE)	Parasite Intensity (± SE)
Cestoda	*Gyrocotyle plana*	Intestine	68.4	1.32 (0.24)	1.92 (0.18)
Monogenea	*Callorhynchicotyle callorhynchi*	Gill	42.1	1.68 (0.78)	4.00 (1.45)
Monogenea	*Callorhinchicola multitesticulatus*	Gill	36.8	0.63 (0.24)	1.71 (0.42)
Isopoda	*Anilocra* sp.	External	21.1	0.32 (0.15)	1.50 (0.29)

2.3.2 Host condition factor

There was no statistical difference between sexes with respect to length (t (17) = -0.3844, p = 0.7063, F = 31.38 cm, M = 33.45 cm) or weight (t (17) = 0.0421, p = 0.97, F = 939 g, M = 921 g). Therefore, the EQN 2.2 was used uniformly across the sampled population. The equation (r^2 = 0.99) showed a length–mass relationship of

$$W = 0.027\ L^{2.91} \qquad \text{(EQN 2.2)}$$

Condition factor (CF) was therefore calculated according to

$$CF = W / 0.027\ L^{2.91} \qquad \text{(EQN 2.1)}$$

www.Fishbase.org recommends *b* values between 2.76 and 3.31 depending on the maturity and sex of the fish measured. With a *b* value of 2.91, the sample caught were within normal growth standards.

2.3.3 Parasite abundance correlations

Normality tests indicated that data was not normally distributed, as expected with parasite count data. Parasite abundance values were correlated with total length, weight and condition factor using the non-parametric Spearman's rank order correlation index. Only *G. plana* abundance was significantly correlated to total length and weight. The correlation coefficient (r_s) for all variables of

G. plana indicate a positive relationship between total length and weight as a function of parasite abundance (Table 2.4).

Table 2.4: Correlation coefficient (r_s) of parasite abundance as a function of total length, weight, and condition factor of host Callorhinchus capensis caught in False Bay, South Africa during 2013. * indicates significance p<0.05.

Parasite Species	**Total Length**			**Weight**			**Condition Factor**		
	r_s	**n**	**p**	**r_s**	**n**	**p**	**r_s**	**n**	**p**
Gyrocotyle plana	0.82	19	< 0.001*	0.80	19	< 0.001*	-0.06	19	0.803
Callorhynchicotyle callorhynchi	0.15	19	0.549	0.15	19	0.550	0.04	19	0.883
Callorhinchicola multitesticulatus	0.06	19	0.820	0.07	19	0.787	-0.15	19	0.551
Anilocra sp.	- 0.18	19	0.464	- 0.08	19	0.746	0.39	19	0.095

2.3.4 Species richness estimation

Randomized, individual-based species accumulation curve (SAC) was drawn for parasites infecting *C. capensis*. SAC, Chao2 and Jackknife1 richness algorithms all estimated a total species richness of 5 parasitic species. The slope of the SAC reached asymptote quickly (within 15 examined hosts) (Figure 2.3).

2.3.5 Parasite biodiversity correlations

Species richness, Shannon Weiner's diversity index (H), Simpsons index (λ) and Pielou's J index (J) values were correlated with total length, weight and condition factor using the non-parametric Spearman's rank order correlation index. Total length and weight showed a significant relationship with all indices. All biodiversity indices indicate a weak negative relationship with condition factor as opposed to positive relationships with total length and weight (Table 2.5).

2.3.6 Sex as a determinant of parasite abundance and prevalence

Parasite species prevalence did not indicate a dependency with sex of *C. capensis* (Table 2.6). However, *C. callorhynchi* abundance did show a dependency on sex (W (36) = 66.5, p = 0.043, female n=8, male n=11).

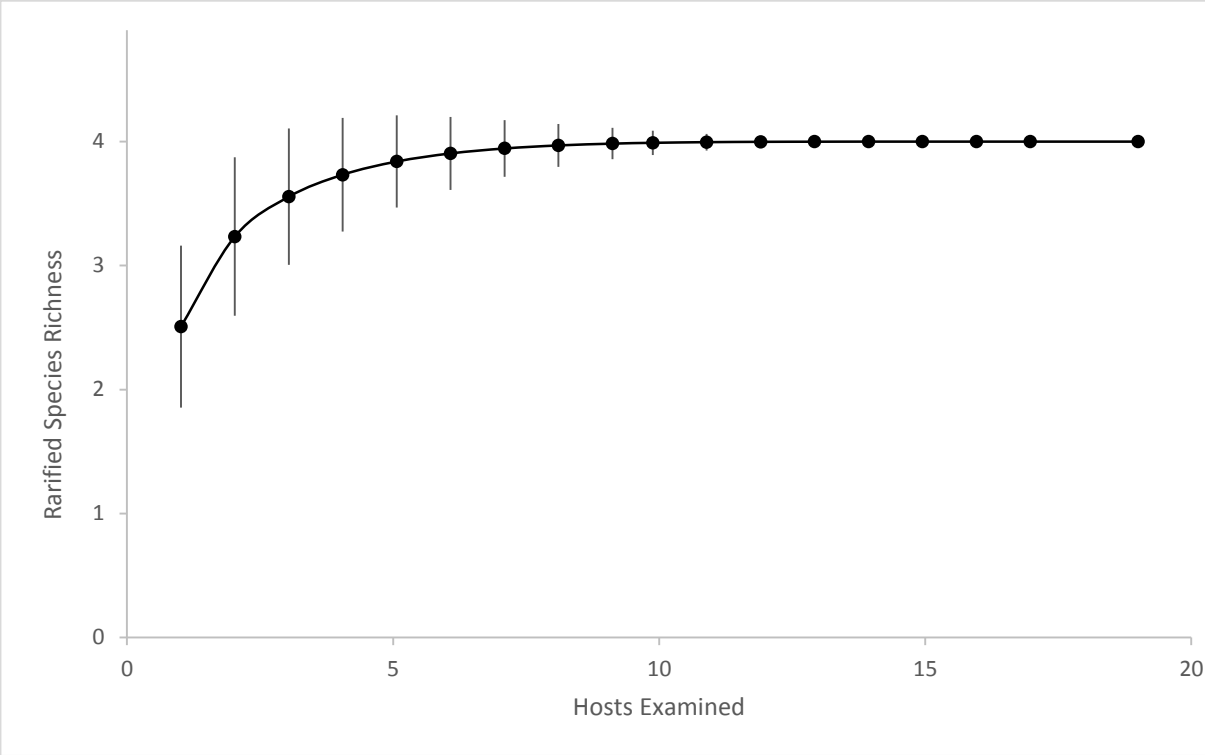


Figure 2.3: Randomized individual based species accumulation curve of parasites infecting *Callorhinchus capensis*. (n=19) caught in False Bay, South Africa during 2013.

Table 2.5: Parasite Species richness, Shannon Weiner's diversity index (H), Simpsons Index (λ) and Pielou's J (J) Index as a function of total length, weight and condition factor of *Callorhinchus capensis* caught in False Bay, South Africa during 2013. * indicates significance p<0.05.

Biodiversity indices	**Total Length**			**Weight**			**Condition Factor**		
	r_s	**n**	**p**	**r_s**	**n**	**p**	**r_s**	**n**	**p**
Species Richness	0.65	19	0.003*	0.65	19	0.002*	-0.08	19	0.734
H	0.62	19	0.005*	0.63	19	0.004*	-0.04	19	0.861
λ	0.63	19	0.004*	0.64	19	0.004*	-0.06	19	0.814
J	0.62	19	0.008*	0.59	19	0.013*	-0.35	19	0.160

Table 2.6: Summary of parasite prevalence (%) and abundance dependence on sex of *Callorhinchus capensis* caught in False Bay, South Africa during 2013 (χ^2 = Chi Squared statistic, U = Mann Whitney U statistic, $p<0.05$).

Parasite Species	**Prevalence**			**Abundance**		
	χ^2	**df**	**p**	**U**	**df**	**p**
Gyrocotyle plana	0.28	1	0.653	41	17	0.827
Callorhynchicotyle callorhynchi	2.36	1	0.167	66.5	17	0.043*
Callorhinchicola multitesticulatus	3.91	1	0.072	63.5	17	0.068
Anilocra sp.	0.13	1	1	47	17	0.772

2.4 Discussion

Reed (2014) highlighted the need for more fundamental research on parasites and their associated fish hosts (both with commercial value and those without) specifically within sub-Saharan Africa. By increasing the knowledge we have on parasite load and expanding the sample areas, we can make more informed management decisions regarding commercially important fish species as well as document previously undiscovered parasitic species. With this increased knowledge on parasitic species integrated with biodiversity indices, it can inform us of parasite dynamics within a population and allow us to get a better picture of the impact of parasites within important ecosystems.

Previous studies suggest at least two other species of parasite that infect *C. capensis*; *Callorhynchicola brachialis* (Beverley-Burton et al., 1993) and an unknown species belonging to the *Branchellion* genus (Bih Awa, 2012). That these two species were not found on the specimens in this study could be due to the small sample size (n=19) or due to a location effect. Both these studies collected specimens off the west coast of South Africa in the cold-temperate Namaqua bioregion. This study utilized inshore specimens caught along the northern coast of False Bay, which is considered part of the warm temperate Agulhas bioregion (Griffiths et al., 2010; Lombard, 2004). There are major differences in ocean conditions between False Bay and the coastal waters off the west coast, with stark differences in biogeography, biodiversity and general characteristics such as productivity, temperature and climatic drivers, to name a few (Sink et al., 2012). These drivers have been shown to have major implications in the distribution of parasitic species and their tendency to infect hosts (Poulin and Morand, 2000).

Gyrocotyle plana was the most prevalent parasite found infecting *C. capensis* as well as the only parasite to correlate significantly with fish length and weight in this study. However, length and weight of a host is intrinsically related and with a range of infection from zero to three parasites, this result may be circumstantial. Larval encysted parasites have been shown to correlate significantly with fish size (e.g. Lo, Morand and Galzin, 1998), which has been attributed to larger hosts requiring more food to satisfy metabolic demands, and ingest more parasitic larva from intermediate hosts. Therefore, the significant relationship could be due to an accumulation of parasites with age.

Gyrocotyle as a genus has very close evolutionary ties to holocephalan sharks around the world (Williams et al., 2009), yet there is little known about the transmission of these parasites. Xylander (1989) suggested a transmission method that involves intermediate hosts due to smaller/younger

sharks having less parasite abundance compared to larger/older sharks. Freer and Griffiths (1993b) also suggest an intermediate host and by examining the stomach contents, concluded it could be a common dietary item due to the high prevalence. If one considers the criteria for selecting an indicator species for False Bay, South Africa, then *G. plana* seems a likely candidate. Both the host and parasite is highly prevalent within False Bay, with defined geographical ranges and strong relationship associated with the host subject.

The species accumulation curve, with its steep slope and early asymptote, suggests the parasitic community structure of *C. capensis* is interactive. Interactive infracommunities are considered to be composed of species with high transmission rates, engaged in strong interspecific interactions, leading to predictable infracommunity structure and high similarity among infracommunities (Dove and Cribb, 2006). Parasitic infracommunities are the sub-populations of parasites living within individual hosts (Poulin, 2001). With parasites showing high prevalence (>20%) the infracommunities seem to be easily predictable and highly similar across the population. All biodiversity indices compliment this finding with species richness, Shannon's diversity index and Simpson's index displaying decreased diversity values. Pielou's J evenness also supports the interactivity of the sample with a value closer to 1, indicating an evenly distributed species diversity across the sampled specimens. The interactive parasite infracommunity also suggests the dynamics with which the host population is being controlled. If the parasitic communities are even across individual hosts, it suggests a host population that is also highly interactive.

There is a lack of biological data for the majority of shark species, including *C. capensis* which makes the development of a management plan for fishery purposes incredibly important (Mann, 2013). By understanding the interactive nature of this species, we can understand how different fishing pressures can affect the species. Breeding is reported to occur throughout the year and females move closer inshore after a 9-12 month gestation to lay oocytes (Freer and Griffiths, 1993a). That sex was not a determinant of parasite abundance supports the interactivity of the population.

There is still much work that needs to be conducted on *C. capensis* and the interaction with its parasites. To name just a few of the future topics; a study into the description of *G. plana* is required, as other holocephalan studies have frequently found two species from the genus *Gyrocotyle*. Only one species from the genus *Gyrocotyle* species has been recorded in *C. capensis*. The life cycle of members of the genus *Gyrocotyle* has still not been described and to understand this life cycle, implications for other species in the ecosystem could come to light. By completing the

life cycle, we can begin to understand the role of complex life cycles within ecosystems. With such a difference in species composition between sites, a more holistic project might need to be established to understand the movement patterns of *C. capensis*. This will have implications on the commercial fishing industry as it could inform us on the stock structure of *C. capensis*. That condition factor did not correlate with parasite statistics or biodiversity indices could be due to a number of reasons, particularly, the variable nature of the condition factor measurement, the small sample size or that sampling was conducted randomly across various seasons. Most of these measurements are highly impacted by seasonal variation within organisms, therefore a seasonal study in parasite prevalence and condition factor is important.

In conclusion, *C. capensis* has a regiment of parasites that remain quite stable throughout the sampled population, with relatively small infection rates and a highly interactive parasite community. *Gyrocotyle plana* is supported as a biological monitoring candidate by fitting many of the requirements, albeit having to compromise on some. This possibility is further explored in Chapter 4. It is clear to see the possibilities with this information and how it can impact the parasite community and provide a few answers to the parasite community dynamics and the biodiversity we experience as a whole within the False Bay marine environment.

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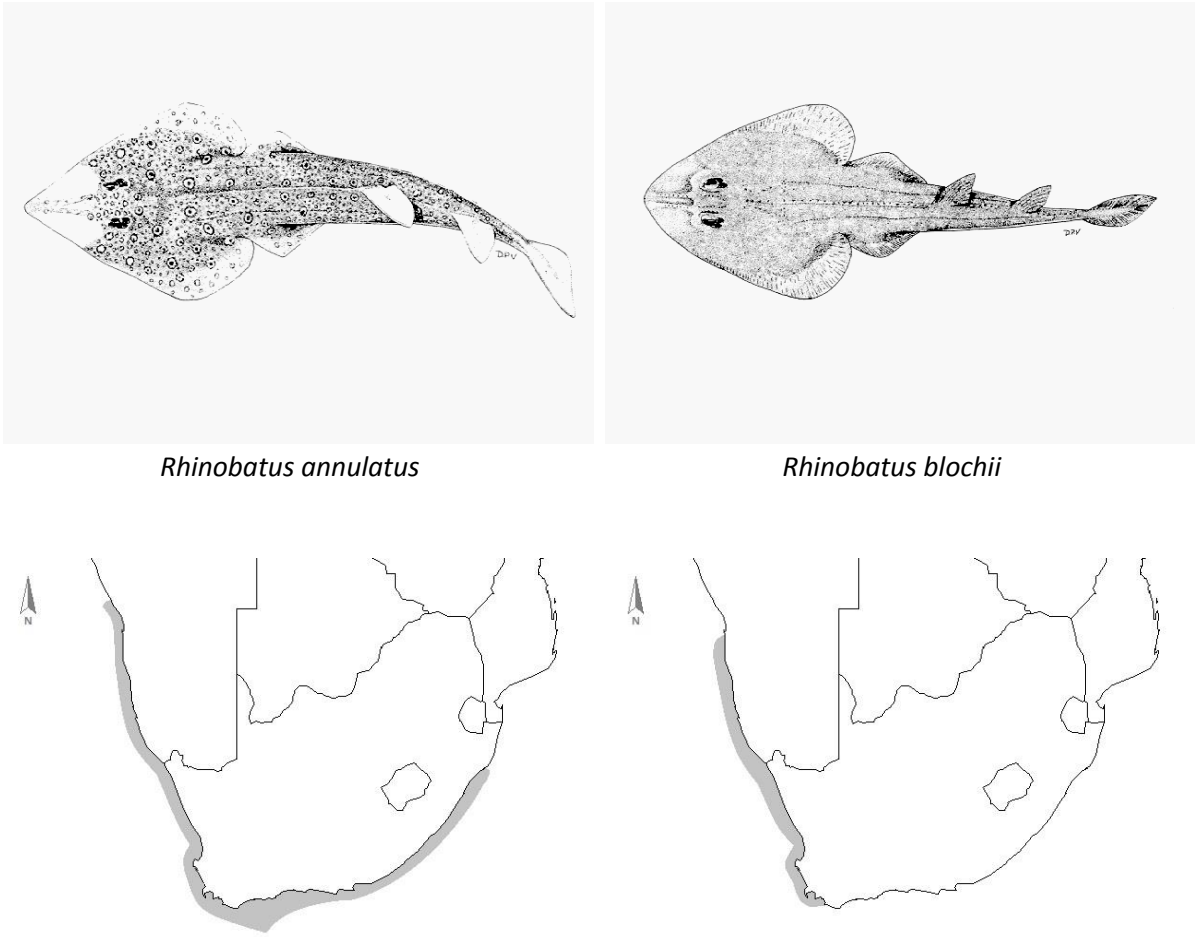
Chapter 3

Parasites of *Rhinobatos annulatus* (Müller and Henle, 1841) and *R. blochii* (Müller and Henle, 1841)

3.1 Introduction

Two rhinobatids of coastal beaches and estuaries, *Rhinobatos annulatus* and *R. blochii* are small elasmobranchs that are found in sandy habitats from shallow surf to inshore trawl grounds. *Rhinobatos annulatus* is endemic from the coast of Angola, Namibia through to the east coast of South Africa (Rossouw, 1984; Smith and Heemstra, 1986), while *R. blochii* is a rare and very little known guitarfish with a narrow distribution limited to ±1,000 km of the western coast of Southern Africa from Walvis Bay, Namibia to Cape Point, South Africa (Fig. 3.1). Species from the *Rhinobatos* genera occur worldwide and consist of 34 species in 5 subgenera. Other species of the genus that occur in the eastern Atlantic include *R. albomaculatus*, *R. cemiculus* and *R. rhinobatos* that all occur as far south as Angola.

Rhinobatos annulatus and *R. blochii's* reproductive strategy includes aplacental vivipary, giving birth to 2 – 10 pups, with adults reaching a maximum length of 140cm and 96cm respectively (Smith and Heemstra, 1986). In South Africa, they are common species found in the Langebaan and Saldanha lagoon system as well as in False Bay (Mann, 2013). Diet includes benthic invertebrates such as small crustaceans, sand mussels and polychaete worms (Smith and Heemstra, 1986). *Rhinobatos annulatus* is a guitarfish with a broad, wedge-shaped snout and pectoral disc. Colouring is tan to dark brown above and white below with numerous small eyespots (dark brown spot ringed with white and a brown margin) on back (Smith and Heemstra, 1986). *Rhinobatos blochii* is a guitarfish with a broadly pointed snout and a broad pectoral disc, plain brown above, and young frequently have white spots (Smith and Heemstra, 1986) (Fig. 3.1).



Rhinobatus annulatus *Rhinobatus blochii*

Figure 3.1: Diagrams of *Rhinobatos annulatus* and *R. blochii* (Smith and Heemstra, 1986) and their distribution along the South African and Namibian coastline.

The species, *R. annulatus* is currently under review and is proposed to be renamed as *Acroteriobatus annulatus* (personal communications, Dr. S. Lamberth and C. Da Silva, 2013 and 2014). This name has been used once in literature (Best et al., 2013), however there has been no published results on www.Fishbase.org or any other reference site, therefore for the purposes of this dissertation, it will be referred as *R. annulatus*, to maintain literature consistency. *Rhinobatos blochii* is a species commonly confused with *R. annulatus* due to its apparent rarity, restricted range within the occurrence area of *R. annulatus* and the continual misidentification by researchers and recreational fishermen. This misidentification has been the cause of historical confusion in identifying the differences between *R. blochii* and *R. annulatus* (as suggested in Mann, 2012). These differences were taken into account and applied through the current study.

3.1.1 Commercial importance of *R. annulatus* and *R. blochii*

In southern Africa, shore anglers catch these species mostly as bycatch, although they are sometimes targeted in catch and release angling competitions. These sharks are also caught as bycatch in beach seine fisheries in False Bay but most are returned alive (Lamberth et al., 1995). Approximately 18t are taken annually as bycatch in the inshore trawl grounds, yet most of which are discarded (Attwood et al., 2011). *Rhinobatos annulatus* and *R. blochii* have very little commertial importance within the South African fishing industry.

Very little research has been done on the biology *R. annulatus* and *R. blochii* (Mann, 2013) largely due to their limited use in commercial industries. Ecologically, however, these sharks are known to play an important role in structuring macroinvertebrate assemblages within the habitats they occupy, such as the Langebaan Lagoon (Harris et al., 1988). Harris et al., (1988) studied the population dynamics and feeding biology of the sand shark which showed seasonal variation in their feeding intensity and that they were concentrated in specific areas to utilize the increased prey biomass. Still very little is known about other aspects of their ecology and in particular, hardly anything is known about their parasitic load as compared to similar species found on the west coast of Africa.

3.1.2 Parasites of *Rhinobatos* spp.

Although South Africa has a rich diversity of elasmobranch species, investigations into their associated parasite faunas and the potential impacts these may have on fish, are rare (Bullard and Dippenaar, 2003; Reed, 2014; Vaughan and Chisholm, 2010). The majority of these publications have focussed on larger, more characteristic species off the east coast of South Africa (Dippenaar, 2009, 2005; Dippenaar et al., 2000, 2008, 2009, 2004, 2001; Dippenaar and Jordaan, 2007, 2006; Dippenaar and Lebepe, 2013; Dippenaar and Olivier, 1999). Although various trematodes, cestodes and monogeneans have been identified from species inhabiting northwest Africa, only five publications (Caira et al., 2013; Hayes et al., 2007; Van As and Basson, 1996; Vaughan and Chisholm, 2010, 2011) of parasites infecting *R. annulatus* are known from South Africa, all of which are taxonomic descriptions of one or more parasitic species (Table 3.1). No previous studies have has been done on *R. blochii* and all parasites found in this study are new host records.

Apps.webofscience.com was searched for publications containing any combination of the terms *rhinobatos* and *parasit*,* published before June 2014. This initial survey yielded 55 publications. Of

these, only 22 publications were relevant to *Rhinobatos* and their parasitic species. These search terms were also placed into Google Scholar. Excluding the references found on Web of Science, a further 13 relevant papers were recorded. Of these 35 relevant papers, the majority of them were broad spread records based studies, measuring various hosts from the ecosystem and recording their parasites (Beveridge et al., 2004; Haseli et al., 2010; Heinz and Dailey, 1974; Izawa, 2011; Neifar et al., 2002, 2001; Palm, 2004; Subhapradha, 1955; Vardo-Zalik and Campbell, 2011). A further few publications focussed on the taxonomic importance of describing new species (Goldstein, 1967; Haseli et al., 2012; Ivanov and Caira, 2012; Kabata, 1993; Kearn, 1978; Tyler, 2001). There was, however, one study that was ecological in nature. Iannacone et al., (2011) measured 36 specimens of *R. planiceps* from the local fish market in Lima, Peru to determine the population dynamics of parasitic metazoans. These researches utilized these data to try and understand the parasite and shark community structure. This and all other papers have been summarized in Table 3.1.

3.1.3 Aims and Objectives

This chapter aims to identify all metazoan parasites that infect *Rhinobatos annulatus* (in False Bay and Saldanha Bay) and *R. blochii* (in Saldanha Bay) and build on the previous yet limited knowledge researchers have described from these species. With this knowledge, a parasitic species that fits biological indicator criteria will be identified and used in heavy metal analysis (see Chapter 4). Individual based species accumulation curves will be constructed for both species, biodiversity indices and condition factor will be calculated and compared to parasitic infection to understand the parasitic community structure present in *R. annulatus* and *R. blochii*.

Table 3.1: Parasite records for species found infecting members of the genus *Rhinobatos*. Table includes location of studies conducted, parasite species, site of infection (SOI), parasite class, and the reference.

Parasite species	Parasite class	SOI	Reference
	***R. annulatus* (South Africa)**		
Echinobothrium dougbermani	Cestode	Spiral valve	(Caira et al., 2013)
Trichodina rhinobatae	Ciliophora	Urogenital tract	(Van As and Basson, 1996)
Gnathia pantherina	Isopoda	Gills	(Hayes et al., 2007)
Pseudoleptobothrium christisoni	Monogenea	Dermal denticles	(Vaughan and Chisholm, 2011)
Neoheterocotyle hargis	Monogenea	Gills	(Vaughan and Chisholm, 2010)
	***R. batillum* (Australia)**		
Paeon australis	Crustacea	External	(Kabata, 1993)
Troglocephalus rhinobatidis	Monogenea	Gills	(Kearn, 1978)
Horricauda rhinobatidis	Monogenea	Gills	(Kearn, 1978)
Calicotyle australis	Monogenea	Cloaca	(Whittington et al., 1989)
	***R. cemiculus* (Tunisia)**		
Mehracotyle insolita	Monogenea	Gills	(Neifar et al., 2002)
Dollfusiella elongata	Cestode	Spiral valve	(Beveridge et al., 2004)
Parachristianella monomegacantha	Cestode	Spiral valve	(Beveridge et al., 2004)
Macrobothridium syrtensis	Cestode	Spiral valves	(Neifar et al., 2001)
	***R. hynnicephalus* (Japan)**		
Dangoka japonica	Crustacea	Gills	(Izawa, 2011)
Eudactylina dasiati	Crustacea	Gills	(Izawa, 2011)
	***R. lentiginosus* (United States of America)**		
Acanthobothrium lentiginosum	Cestode	Spiral valve	(Vardo-Zalik and Campbell, 2011)
	***R. leucorhynchus* (Mexico)**		
Echinobothrium hoffmanorum	Cestode	Spiral valve	(Tyler, 2001)
Echinobothrium rayallemangi	Cestode	Spiral valve	(Tyler, 2001)
	***R. planiceps* (Peru and Chile)**		
Eudactylina peruensis	Copepoda	External	(Luque and Farafan, 1991)
Stibarobdella moorei	Hirudinea	External	(Iannacone et al., 2011)
Ommatokoita elongata	Copepoda	Eyes, external	(Iannacone et al., 2011)
Anoplocotyloides papillatus	Monogenea	Gills	(Iannacone et al., 2011)
Anoplocotyloides chorrillensis	Monogenea	Gills	(Iannacone et al., 2011)
Rhinobatonchocotyle pacifica	Monogenea	Gills	(Oliva and Luque, 1995)
Rhinebothrium rhinobati	Cestode	Spiral valve	(Dailey and Carvajal, 1976)
Parachristianella monomegacantha	Cestode	Spiral valve	(Palm, 2004)
Prochristianella heteracantha	Cestode	Spiral valve	(Dailey and Carvajal, 1976)
Acanthobothrium olseni	Cestode	Spiral valve	(Iannacone et al., 2011)
Proleptus carvajali	Nematode	Spiral valve	(Iannacone et al., 2011)
Proleptus acutus	Nematode	Spiral valve	(Dailey and Carvajal, 1976)

Table 3.1: Continued…

Parasite species	Parasite class	SOI	Reference
	***R. productus* (Mexico)**		
Pseudcchristianello nudiscula	Cestode	Spiral valve	(Campbell and Beveridge, 2006)
Prochrisianella fragilis	Cestode	Spiral valve	(Heinz and Dailey, 1974)
Acanthobothrium rhinobati	Cestode	Spiral valve	(Alexander, 1953)
Acanthobothrium olseni	Cestode	Spiral valve	(Dailey and Mudry, 1968)
Acanthobothrium robustum	Cestode	Spiral valve	(Alexander, 1953)
Eutetrarhynchus schmidti	Cestode	Spiral valve	(Heinz and Dailey, 1974)
Lacistorhynchus dollfusi	Cestode	Spiral valve	(Palm, 2004)
Parachristianella monomegacantha	Cestode	Spiral valve	(Heinz and Dailey, 1974)
Anaporrhutum euzeti	Gorgoderidae	Spiral valve	(Curran et al., 2003)
	***R. punctifer* (Iran)**		
Trygonicola macroporus	Cestode	Intestine	(Haseli et al., 2010)
Eutetrarhynchus platycephali	Cestode	Intestine	(Haseli et al., 2010)
Pseudochristianella southwelli	Cestode	Intestine	(Haseli et al., 2010)
Dollfusiella sp.	Cestode	Intestine	(Haseli et al., 2010)
Echinobothrium persiense	Cestode	Spiral valve	(Haseli et al., 2012)
	***R. rhinobatos* (Tunisia)**		
Calicotyle vicina	Monogenea	Cloaca	(Neifar et al., 2002)
Neoheterocotyle ktarii	Monogenea	Gills	(Neifar et al., 2001)
Echinobothrium euterpes	Cestode	Spiral valve	(Neifar et al., 2001)
Dollfusiella elongata	Cestode	Spiral valves	(Beveridge et al., 2004)
Parachristianella monomegacantha	Cestode	Spiral valves	(Beveridge et al., 2004)
Hysterothylacium aduncum	Nematode	Spiral valves	(Genc et al., 2005)
	***R. schlegelii* (India and Japan)**		
Dangoka japonica	Crustacea	Gills	(Izawa, 2011)
Eudactylina dasiati	Crustacea	Gills	(Izawa, 2011)
Acanthobothrium southwelli	Cestode	Spiral valves	(Goldstein, 1967)
Echeneibothrium filamentosum	Cestode	Spiral valves	(Subhapradha, 1955)
Orectolobicestus chiloscylli	Cestode	Spiral valves	(Subhapradha, 1955)
	***R. typus* (Australia)**		
Merizocotyle icopae	Monogenea	Gills	(Chisholm and Whittington, 2000)
Troglochephalus Rhinobatidis	Monogenea	Gills	(Chisholm and Whittington, 2000)
Neoheterocotyle rhinobatidis	Monogenea	Gills	(Chisholm and Whittington, 2003)
Prochristianella spinulifera	Cestode	Spiral valve	(Beveridge and Jones, 2000)
Echinobothrium chisholmae	Cestode	Spiral valve	(Jones and Beveridge, 2001)
	***R. thouin* (Malasia)**		
Empruthotrema dasyatidis	Monogenea	Gills	(Chisholm and Whittington, 2005)
Mycteronastes icopae	Monogenea	Gills	(Chisholm and Whittington, 2005)
Echinobothrium sematanense	Cestode	Spiral valve	(Ivanov and Caira, 2012)

3.2 Methods

3.2.1 Collection and Dissection protocol

Samples of *R. annulatus* were collected from commercial beach seine net fishermen off the coast between Sunrise Point and Strandfontein Point in False Bay. *Rhinobatos annulatus* and *R. blochii* are both present in Saldanha Bay, and with the aid of a beach trek net (50m long and 1.5m deep) with a mesh size of 1 cm, they were hauled in and collected. All samples were collected intermittently between March 2013 and April 2014 (Table 3.2). A total of 19 *R. annulatus* and 17 *R. blochii* were collected, ranging in size from 350 mm – 767 mm and 549 mm – 956 mm respectively. Samples were then transported to the University of Cape Town and frozen at -20^{o}C till processing.

Table 3.2: Collection details of samples of *Rhinobatos annulatus* (RA) and *Rhinobatos blochii* (RB).

Year	**Date of capture**	**Species**	**Location**	**Sample size (n)**	**Size Range (mm)**
2013	March	RA	Saldanha Bay	3	713 - 767
2013	June	RB	Saldanha Bay	13	579 - 956
2013	November	RA	False Bay	15	350 - 751
2014	March	RA	Saldanha Bay	1	610
2014	April	RB	Saldanha Bay	4	549 - 674

Dissection protocol is similar to that of Chapter 2 for *Callorhinchus capensis.* Biological data such as sex, weight to the nearest gram (g) and length (total and standard) to the nearest millimetre (mm) were recorded. Relative condition factor was calculated utilizing the equations from Froese, (2006) and Le Cren, (1951). (See equations 2.1 and 2.2)

$$CF = W / a L^{b} \qquad \text{EQN 2.1}$$

Where W = weight (g), L = total length (cm). The exponent *a* and *b* is derived from the length–mass relationship which is described by:

$$W = a L^{b} \qquad \text{EQN 2.2}$$

These values were then compared to www.Fishbase.org to see if calculated *b* variables were within range of species norm.

A survey of the parasitic fauna on both species was conducted as per the method explained in Chapter 2 for *Callorhinchus capensis*, which is outlined by MacKenzie and Abaunza, (2005). Once an external examination of macro parasites is complete, gills were removed and separated into petri dishes and examined with a dissecting microscope (Leica EZ4). The shark was then eviscerated and kidney, liver, muscle, gall bladder, and gonad samples were smeared and examined at 40 x magnification (Leica Icc50, DM 750). Any parasitic species discovered during this process had their abundance recorded and were placed in 10% formalin for preservation.

3.2.2 Statistical analysis

Parasite summary statistics were calculated following the guidelines set out by Bush *et.al.* (1997) and outlined in Chapter 2 of this thesis. Prevalence, mean intensity and mean abundance were calculated for each parasite species. Species Accumulation Curves were utilized to determine the basic information used to validate richness comparisons and were drawn for both species of shark. Biodiversity indices such as rarefied species richness, Shannon Weiner's Diversity index (H), Simpsons index (λ) and Pielou's J (J) index were calculated with the same methods mentioned in Chapter 2. Correlations between biological measures and diversity indices were attempted with the use of Spearman's rank order correlation index to confirm statistical significance at 95% ($p<0.05$). The Spearman's rank order correlation or Spearmans rho (r_s) is a non-parametric measure of statistical dependence between two variables and is outlined in more detail in Chapter 2. Chi squared (χ_2) and Mann Whitney U statistical analyses were used to test for the significant affect sex and location may have on the prevalence and abundance of parasitic infection. The use of parametric/non-parametric tests were chosen by testing the normality and homogeneity of the variables by frequency distributions, Q-Q plots and the Shapiro-Wilk test.

All analyses, were conducted in either Microsoft Excel (2013) or R 2.15.1 (R Core Team, 2012), with the use of R packages Vegan (Oksanen *et. al.*, 2012) for rarefaction and calculation of biodiversity indices.

3.3 Results

3.3.1 Summary statistics

Four metazoan parasites were found infecting a sample total of 36 specimens of *R. annulatus* and *R. blochii* (Tables 3.3 and 3.4, Figure 3.2)). These included two species of Nematoda found infecting the stomach (*Proleptus obtusus*) and encysted in the kidneys (*Ascaris* sp.) and a copepod species (*Clavelottis* sp.). *Proleptus obtusus* was the most prevalent species infecting 31.6 % and 29.4 % of the samples respectively. In both host species, *P. obtusus* was the most abundant (1 ± 0.37; 3.68 ± 2.76) with the highest mean infection intensity (3.17 ± 0.4; 14 ± 1.5) (Table 3.3). The cestode (*Trilocularia* sp.) was only found in three of the *R. annulatus* specimens, all from False Bay.

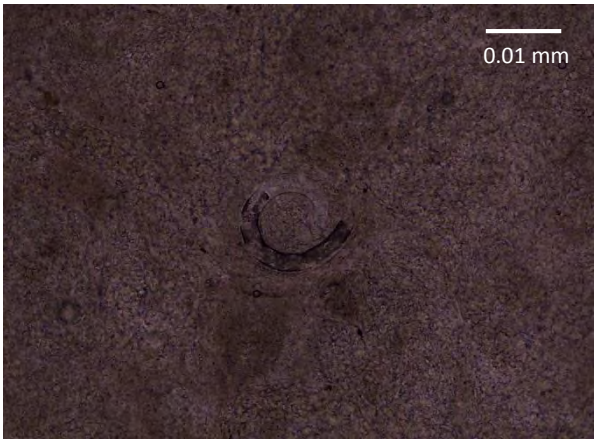
Table 3.3: Summary statistics for parasites found infecting *Rhinobatos annulatus* caught in False Bay and Saldanha bay, South Africa from March 2013 to April 2014 (n=19). (SOI = site of infection)

Parasite Class	**Parasite Species**	**SOI**	**Prevalence (%)**	**Parasite Abundance (± SE)**	**Parasite Intensity (± SE)**
Nematoda	*Proleptus obtusus*	Stomach	31.6	1 (0.37)	3.17 (0.4)
Nematoda	*Ascaris* sp. (cyst)	Kidney	5.3	0.05 (0.05)	1
Cestoda	*Trilocularia* sp.	Spiral Valve	15.8	0.16 (0.9)	1
Copepoda	*Clavelottis* sp.	Gill arch	10.5	0.11 (0.07)	1

Table 3.4: Summary statistics for parasites found infecting *Rhinobatos blochii* caught in Saldanha bay, South Africa from March 2013 to April 2014 (n=17). (SOI = site of infection)

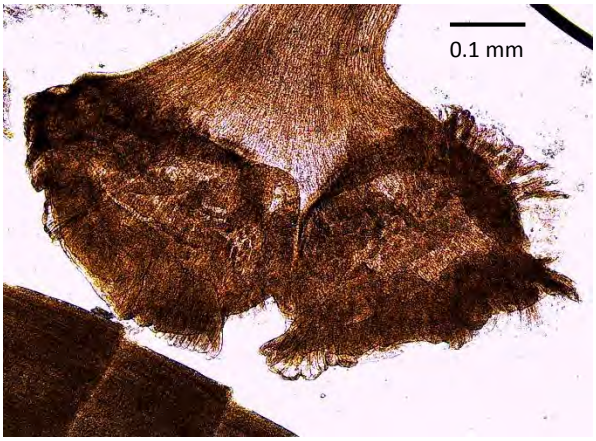
Parasite Class	**Parasite Species**	**SOI**	**Prevalence (%)**	**Parasite Abundance (± SE)**	**Parasite Intensity (± SE)**
Nematoda	*Proleptus obtusus*	Stomach	29.4	3.68 (2.76)	14 (1.5)
Nematoda	*Ascaris* sp. (cyst)	Kidney	11.8	0.37 (0.31	3.5 (8.32)
Copepoda	*Clavelottis* sp.	Gill arch	5.9	0.05 (0.06)	1

Proleptus obtusus (40x)



Ascaris sp. (100x)

Clavelottis sp.



Trilocularia sp. (40X)

Figure 3.2: Metazoan parasites found infecting *Rhonobatos annulatus* and *R. blochii* caught in False Bay and Saldanha Bay, South Africa from March 2013 to April 2014.

3.3.2 Calculating Condition factor

EQN 2.2 (r^2= 0.99, for both regressions) was calculated separately for each species and applied uniformly across that species as there was no statistical distinction between the lengths (RA: t(17) = -0.577, p = 0.5712 , F = 47.31 cm, M = 51.06 cm; RB: t (15) = -0.3844, p = 0.7063, F = 73.29 cm, M = 75.45 cm) or weights (RA: t(17) = 0.204, p = 0.841, F = 824.5 g, M = 770.6 g; RB: t (15) = 0.0421, p = 0.97, F = 2064 g, M = 1932 g) of either sex within the species.

$W = 0.0031\ L^{3.04}$ for *R. annulatus* (EQN 2.2)

$W = 0.0038\ L^{3.13}$ for *R. blochii*

Condition Factor (CF) was therefore calculated according to:

$CF = W / 0.0031\ L^{3.04}$ for *R. annulatus* (EQN 2.1)

$CF = W / 0.0038\ L^{3.13}$ for *R. blochii*

www.Fishbase.org recommends *b* values between 2.86 - 3.24 for *R. annulatus* and 2.89 - 3.31 for *R. blochii* depending on the maturity and sex of the fish measured. With a *b* value of 3.04 and 3.13 respectively, the samples caught were within normal growth standards.

3.3.3 Parasite abundance correlations

Normality tests indicated that data was not normally distributed, as expected with parasite count data. The non-parametric Spearman's rank order correlation index was used to test if there was a correlation between parasite abundance values and total length, weight and condition factor of each parasitic species. No correlations were found.

3.3.4 Species richness estimation

Randomized, individual-based species accumulation curves (SAC) were drawn for parasites infecting *R. annulatus* and *R. blochii*. SAC, Chao2 and Jackknife1 richness algorithms all estimated a total species richness of five parasitic species that infect both species if considered as one population (Fig 3.3 a). The slope of the SAC for both species is gradual and a late asymptote is reached (only by 30 examined hosts). The Individual SAC's show varying estimates. *Rhinobatos annulatus* is predicted in having five parasitic species while *R. blochii* is predicted in having three parasitic species. Both these curves have gradual initial slopes that don't reach their respective asymptotes (Fig 3.3 b).

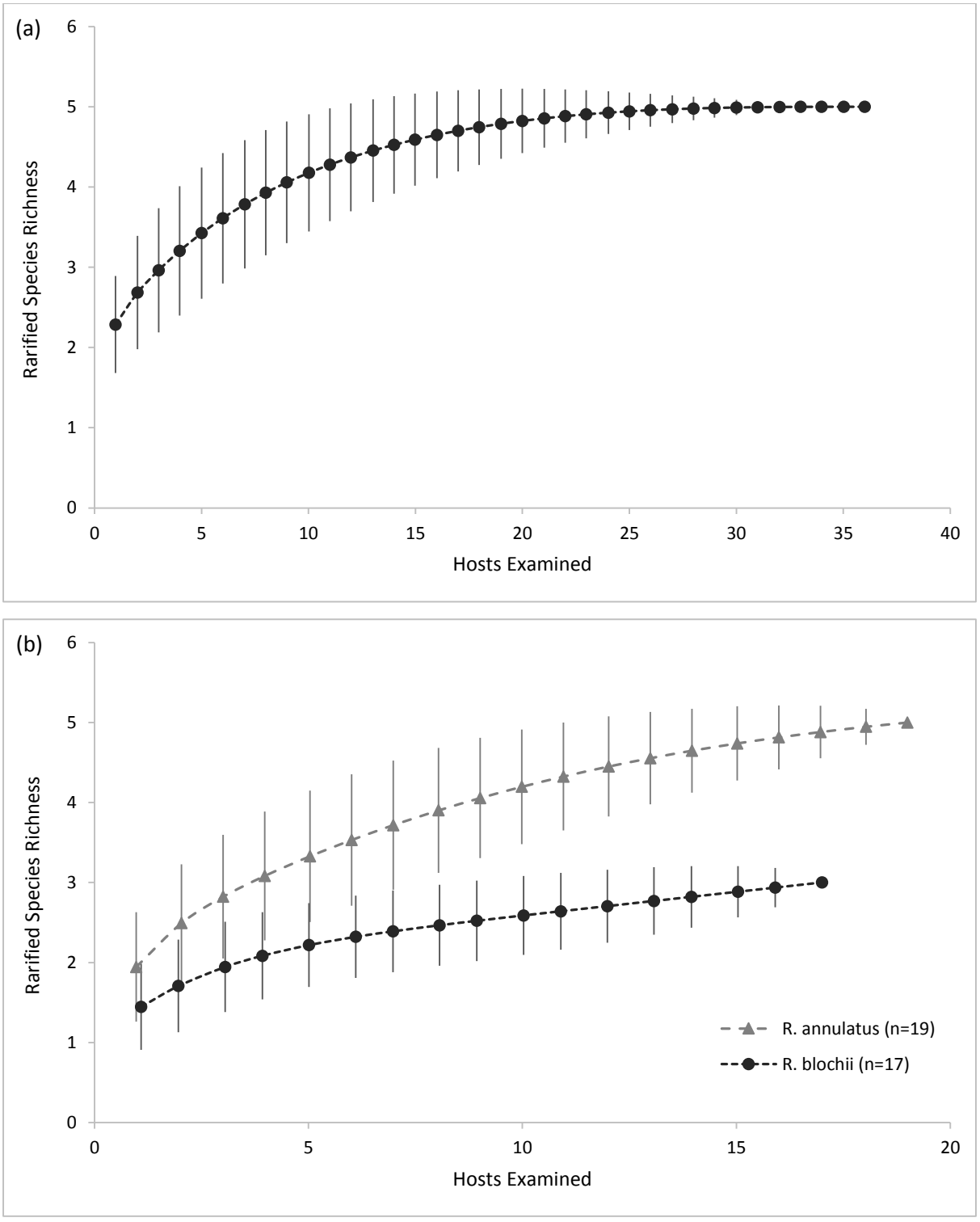


Figure 3.3: Randomized individual based species accumulation curves for parasites infecting both species (*a*) (n=36) and *R. annulatus* and *R. blochii* (b) caught in False Bay and Saldanha bay, South Africa from March 2013 to April 2014.

3.3.5 Parasite biodiversity correlations

Parasite species richness, Shannon Weiner's diversity index (H) and Simpsons Index (λ) all correlated significantly with total length and weight of *R. annulatus* (Table 3.5). Simpsons Index was the only biodiversity variable not to indicate a positive relationship with total length, weight and condition factor. *Rhinobatos blochii* total length, weight and condition factor did not significantly correlate with any of the Biodiversity indicies (Table 3.6).

Table 3.5: Parasite species richness, Shannon Weiner's diversity index (H), Simpsons Index (λ) and Pielou's J (J) Index as a function of total length, weight and condition factor for *Rhinobatos annulatus* caught in False Bay and Saldanha bay, South Africa from March 2013 to April 2014 (n=19). (* indicates significance p<0.05)

Biodiversity indices	**Total Length**		**Weight**		**Condition Factor**	
	r_s	**p**	**r_s**	**p**	**r_s**	**p**
Species Richness	0.80	>0.001*	0.78	>0.001*	0.28	0.243
H	0.53	0.020*	0.53	0.018*	0.38	0.109
λ	-0.83	>0.001*	-0.81	>0.001*	-0.2	0.4
J	0.74	0.001*	0.71	0.002*	0.46	0.071

Table 3.6: Parasite species richness, Shannon Weiner's diversity index (H), Simpsons Index (λ) and Pielou's J (J) Index as a function of total length, weight and condition factor for *Rhinobatos blochii* caught in Saldanha bay, South Africa from March 2013 to April 2014 (n=17). (* indicates significance p<0.05)

Biodiversity indices	**Total Length**		**Weight**		**Condition Factor**	
	r_s	**p**	**r_s**	**p**	**r_s**	**p**
Species Richness	0.19	0.457	0.17	0.6	-0.04	0.886
H	0.36	0.159	0.36	0.159	0.33	0.192
λ	-0.07	0.788	-0.01	0.979	0.18	0.492
J	0.5	0.117	0.5	0.117	0.35	0.289

3.3.6 Sex and location as a determinant of parasite abundance and prevalence

Parasite prevalence and abundance did not correlate with sex, for either species of host (Table 3.7 and table 3.8). *Clavelottis* sp. prevalence and abundance (U (17) = 15, p = 0.006) indicated a significant difference (χ^2 (1) = 8.38, p = 0.039 ; U (17) = 15, p = 0.006) between the locations of *R. annulatus*. The abundance of *P. obtusus* infecting *R. annulatus* (U (17) = 12.5, p = 0.039) also showed a significant difference (U (17) = 12.5, p = 0.039) between locations. Otherwise there was no other determinant of parasite abundance between the parasite species and the different locations of the host species (Table 3.7 and table 3.8).

Table 3.7: Chi squared test (χ^2, df = 1) of parasite prevalence and Mann-Whitney U test of parasite abundance dependency on sex and location for *Rhinobatos annulatus* caught in False Bay and Saldanha bay, South Africa from March 2013 to April 2014. (* indicates significance p<0.05)

Parasite Species	**Sex**					**Location**				
	χ^2	**p**	**U**	**df**	**p**	**χ^2**	**p**	**U**	**df**	**p**
Proleptus obtusus	0.69	0.627	52	17	0.519	4.42	0.079	12.5	17	0.039*
Ascaris sp. (cyst)	1.17	0.460	40	17	0.343	0.28	1	32	17	0.698
Trilocularia sp.	0.53	0.596	39.5	17	0.518	0.95	0.561	36	17	0.384
Clavelottis sp.	2.01	0.462	54	17	0.192	8.38	0.039*	15	17	0.006*

Table 3.8: Chi squared test (χ^2) of parasite prevalence andMann-Whitney U test of parasite abundance dependency on sex for *Rhinobatos blochii* caught in Saldanha bay, South Africa from March 2013 to April 2014. (* indicates significance p<0.05)

Parasite Species	**Sex**					
	χ^2	**df**	**p**	**U**	**df**	**p**
Proleptus obtusus	1.89	1	0.274	23	15	0.236
Ascaris sp. (cyst)	1.23	1	0.530	39	15	0.324
Clavelottis sp.	0.58	1	1	36	15	0.538

3.4 Discussion

Previous parasitic surveys conducted on *R. annulatus* indicate at least five other parasite species that infect this host; particularly two monogenean, a cestode, an isopod and a ciliophoran (Table 3.1), which have not been recorded in this study (Caira et al., 2013; Hayes et al., 2007; Van As and Basson, 1996; Vaughan and Chisholm, 2010, 2011). Majority of these studies were conducted on *R. annulatus* specimens that were caught at depth 35.2 - 192.9m on the Agulhus Bank off the south coast of South Africa, except for Hayes *et al.*, (2007), who caught specimens by rod and line off the shore at De Hoop Nature reserve, also situated on the south coast. It is well established that differing environmental drivers control the distribution of parasites and their tendency to infect their hosts (Poulin and Morand, 2000) and could explain the significant differences experienced with *P. obtusus* and *Clavelottis sp.* with respect to their location. With *R. annulatus* having limited movement and considering the variable oceanic conditions across the South African bioregions (Sink et al., 2012), the differing parasite assemblages and low prevalence's found across these studied populations are expected.

The *R. annulatus* and *R. blochii* species caught in this survey displayed a limited amount of infection by parasites, with a maximum prevalence of only 31.6% and 29.4%. Nematoda is one of the most diverse and successful groups in the animal kingdom and studies have found 100% prevalence in their hosts (McLachlan, 2011; Yeld, 2009). *Proleptus obtusus* is a generalist parasite known to infect various species along the South African coast (McLachlan, 2011). That this common species it did not have a high infection rate in *R. annulatus* and *R. blochii*, which is contradictory to the literature. McLachlan, (2011), measured the phylogeography of the catshark, *Haploblepharus pictus* and its parasite *Proleptus obtusus* to build a picture of the population dynamics of this charismatic shark. Specifically with *P. obtusus*, it was found that the parasite is more host specific than previously considered for this generalist species. This limited prevalence in *R. annulatus* and *R. blochii* could be indicative of a host species that has established strong defences against infection or it could be indicative of a population that is resident and considered an isolationist species. The limited amount of infection recorded in this study could also be a result of limited sample size (n=19 and n=17 respectively), confirming the need for a more extensive study into these two *Rhinobatid* species

Isolationist infracommunities are considered to be composed of species with low transmission rates, engaged in few or weak interspecific interactions, leading to unpredictable infracommunity structure and low similarity between infracommunities (Dove and Cribb, 2006). Within *R. annulatus* and *R.*

blochii's parasitic assemblage, all parasite species show low prevalence values (< 31%). Species Accumulation Curves show a gradual initial slope with the curve reaching a late asymptote. There was significant correlations with total length and weight and their associated biodiversity indices (species richness, Shannon Weiner's diversity index and Simpsons Index), however, the correlations were only experienced in *R. annulatus*. These results and the variable parasite infracommunities across *R. annulatus* and *R. blochii*'s range support this isolationist hypothesis.

Qualifying the infracommunity of *R. annulatus* and *R. blochii* as isolationist, allows analysis of individual parasitic species and predict their role in the parasitic assemblage. For example, the *Trilocularia* sp. falls under the tetraphyllidean order and is largely host specific, mainly infecting elasmobranchs with a few species infecting the holocephelan sharks (Rohde, 2005). Studying the prevalence data and searching the community ecology indices, we are able to predict the structure of the community with reference to the *Trilocularia* sp. This can then inform management procedures of these sharks and their associated parasites and suggests potential threats to their existence. Particularly for the *Trilocularia* sp. found in this study, it seems to occur in a very limited area within False Bay as there was no other record for it outside this region. It is therefore, recommended that within False Bay, the city of Cape Town maintain their marine protected areas, not just to conserve this common shark species, but more importantly the uncommon *Trilocularia* sp.

The nematode, *P. obtusus*, demonstrates itself as a potential indicator species due to it having the highest prevalence and infection intensity found across the two study hosts (30.6 %). It also showed the highest abundance which also indicated a significant difference in abundance of parasites between hosts found at the two study areas. As previously mentioned, *P. obtusus* is a common species found off the coast of South Africa recorded from the dark shyshark, *Haploblepharus pictus*, the puffadder shyshark, *H. edwardsii* and the pyjama shark, *Poroderma africanum* (Yeld, 2009).

Although *R. annulatus* and *R. blochii* are similar species occurring in the same area, there is a significant difference in the size of these hosts, with *R. blochii* being larger than its counterpart. This information was utilized to calculate condition factor separately. The condition factor did not correlate with parasite abundance or with any of the biodiversity indices. Condition factor can provide information on the general condition of fish in the habitat, how they live and various other important physiological traits. Unfortunately, it is also highly dependent on many biotic and abiotic

factors that if not monitored in conjunction, can cause too much variability. Therefore not having a correlation could be attributed to environmental, seasonal, physiological or any other interactions.

Rhinobatos annulatus is labelled as being a major player in the structuring of benthic communities, which can drastically change ecosystems (Harris *et al.*, 1988). Yet, even with high abundances in local coastal areas, there has been little attention from research entities. However, studying this unique species provides a unique window into the population dynamics and allows us to contribute to the knowledge we have of our ecosystems. By increasing our knowledge, we can make more informed decisions on maintaining the biodiversity within these ecosystems, not just for the free living organisms, but the parasitic organisms that have shown to play a much larger role in these ecosystems.

In conclusion, *R. annulatus* has been more thoroughly studied in terms of their parasites compared to *R. blochii* (Caira et al., 2013; Hayes et al., 2007; Van As and Basson, 1996; Vaughan and Chisholm, 2010, 2011). With the parasite assemblage of these two species qualified as isolationist, a more intensive and fine grain sampling strategy is required to understand the parasite diversity we experience in Saldanha and False Bay. By continuing to grow the parasite records, we can understand the parasite assemblage structure and derive the structure of the host community, which in the case of *R. annulatus*, is extremely isolationist. There is still a plethora of future work to be conducted on these two species of shark, including expanding the sampling to establish the home ranges of the parasites within the home ranges of their hosts. There is also a need for taxonomic studies to be conducted as many of these parasites have not been identified to species level (e.g.: *Trilocularia* sp.).

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Chapter 4

Fish parasites as bio-indicators of heavy metals in South African marine ecosystems

"… there is only one pollution, because every single thing, every chemical whether in the air or on land will end up in the ocean." - Jacques Cousteau (1971).

4.1 Introduction

Land based pollution is said to account for 77% of all pollution impacting our oceans (Williams, 1996). This is derived from human settlements, land use, construction of coastal infrastructure, agriculture, forestry, urban development, tourism and industry (Johnson, 1993). It is therefore these land based sources of pollution that are impacting coastal areas worldwide, which are having causative effects on commercial coastal and marine fisheries (Islam and Tanaka, 2004). This pollution problem, as described by Williams (1996), is further characterized by interconnectedness, complicated interactions, uncertainty, conflicts and constraints, making it difficult to control the problem. Moreover, because scientific knowledge on marine pollution is patchy, these knowledge gaps have been identified as one of the major problems in introducing effective management strategies for the control of pollution (Islam & Tanaka, 2004). It is only through measuring and monitoring marine pollution, that we will see sustained management and eventual conservation of aquatic resources.

Trace elements have become important pollutants to measure and monitor for a number of reasons. Firstly, for the past decade they have been shown to increase dramatically worldwide, particularly from land based activities (Robinson and Avenant-Oldewage, 1997). Secondly, they have long residence times and therefore have high mobility across marine ecosystems and within organisms (Goldberg, 1995; Islam and Tanaka, 2004). Finally, marine organisms are particularly susceptible to these pollutants and there is support for the strong bio-magnification up the food web (Sadiq, 1992). Trace elements are divided into three groups, depending on their essentiality: (a) those known to be essential for normal metabolic functioning within organisms, (b) those that have beneficial metabolic effects but have not been shown to be essential, and (c) those that occur widely in living organisms but seem to be only incidental contaminants and are not beneficial to the organism (Kapustka et al., 2003). It is this distinction that allows for effective risk assessment of these toxic

compounds in organisms and the surrounding environment. The role that these essential metals (cobalt, chromium, copper, manganese, nickel, selenium and zinc) play in biological functions include helping in physiological processes, development of respiration and reproductive function and being an integral part of protein and enzyme functionality (Bryan, 1971). However, like the non-essential elements, the essential and beneficial elements are toxic at high concentrations and can have deleterious effects on an organism's health. It is due to these responses and the nature of trace metals in the marine system, that these organisms have received increasing attention in the monitoring of pollution.

4.1.1 Heavy metal pollution research in South African marine environments

Marine pollution studies that have been conducted in South African coastal waters that have utilized bio-indicator species, have mainly focussed on the metal content of mussels and abalone. However, these studies were conducted 30 to 40 years ago, in the 1970's and early 1980's, as part of the National Marine Pollution Monitoring Programme (Cloete and Oliff, 1976; Cloete and Watling, 1981; Gardner et al., 1983). In 1985, the South African National Committee for Oceanographic Research (SANCOR) developed the Marine Pollution Research Programme (MPRP) as a framework for pollutant research (Hennig, 1985). As part of the MPRP, the Mussel Watch Programme was established, but the data have not been made available to the public until recently (Atkinson et al., 2006; Sparks et al., 2014). Sparks et al. (2014) concluded that metal concentrations along the Cape Peninsula are highly variable but have remained constant over the study period. Apart from the Mussel Watch Programme, there has been a sharp decline in pollution research since the 1990's. The majority of research projects currently being undertaken in South Africa are extremely diverse in their objectives and practice (O'Donoghue and Marshall, 2003). These studies are linked to impact assessment studies by local authorities (municipalities) and exposure studies on local fauna, sediment and water quality (Wepener and Degger, 2012). The majority of these studies are conducted by University research groups, for example Reinecke et al., (2012) out of Stellenbosch University, who measured cadmium body loads of four intertidal invertebrates within False Bay. This research was published out of a PhD conducted by Mdzeke, (2004).

4.1.2 Biological indicators

Biological monitoring can be defined as "…the use of biological responses by organisms in effected environments to evaluate changes in these environments with the intent to use this information in a quality program." (Sures, 2005). The use of biological indicator organisms to define areas of trace metal pollution appears most attractive, as these organisms not only concentrate metals from water, allowing inexpensive and relatively simple analysis, but they may also represent a moving time-averaged value for the relative biological availability of metals at each site studied (Rainbow and Phillips, 1993). The pathogenic effects of metals on organisms is associated with their inhibition of enzymatic systems, and in high concentrations, they act on the surface tissue of organs as protein precipitants (Sures, 2001). Once absorbed into the system, they concentrate in protein rich organs (e.g., lymphocytes, liver, muscle etc.), and in high concentrations, cause a variety of morphological, inhibitory and behavioural responses (See Bryan et al., 1979 for a comprehensive review on the impact of metals on marine organisms). It is, therefore, these biological indicator organisms that provide valuable information about the chemical state of their environment.

As top predators, sharks have been shown to accumulate higher concentrations of metals than their counterparts further down the food web. This increased accumulation is attributed to biomagnification (accumulating metals from food) due to their hierarchy in the food chain (Al-Reasi et al., 2007; Marcovecchio et al., 1991). *Apps.webofscience.com* was searched for publications containing any combination of the terms *shark**, *accumulation,* and *metal** published before December 2014. The results indicate a variety of studies conducted worldwide: Australia and New Zealand (Bellamy and Hunter, 1997; Walker et al., 2014), Brazil (Cascaes et al., 2014; Ferreira et al., 2004), Mexico (Hurtado-Banda et al., 2012), the Mediterranean (Blanco et al., 2008; Branco et al., 2007; De Boeck et al., 2010; Kannan et al., 1996; Storelli et al., 2002, 2011), Oman (Al-Mughairi et al., 2013; Golestaninasab et al., 2014; Malek et al., 2007) and the USA and Canada (Lyons et al., 2013; Rumbold et al., 2014) just to name a few. Most of these studies focus on a combination of arsenic, cadmium, copper, manganese, mercury, and zinc with a select few studies focussing on the health risks on humans if contaminated shark meat is consumed (For South African references see Bosch et al., 2013; Erasmus, 2004).

However, as useful as sharks are in heavy metal bioaccumulation studies, only two papers have been published on the usefulness of their parasites as indicators of heavy metal accumulation (Golestaninasab et al., 2014; Malek et al., 2007). What these publications demonstrate is the ability

of parasites to accumulate metals up to 455 times that of the surrounding host tissues, supporting sharks and their parasites as perfect potential bio-indicator species.

4.1.3 Aims and Objectives

This chapter aims to identify whether *Callorhinchus capensis*, *Rhinobatos annulatus* and *R. blochii* and their parasites could be used as biological indicators for heavy metal accumulation in two South African marine ecosystems. The data will be useful in establishing whether there is a difference in accumulation between the various shark species and whether there is a difference in metal concentrations between the two localities. Furthermore, the data will establish in which host organs the majority of metals are being accumulated and allow us to predict the effect of the metal concentrations on the parasites, their shark hosts and eventually the population within the ecosystem.

4.2 Methods

4.2.1 Sample Collection and preparation

Samples of *C. capensis* and *R. annulatus* were collected from commercial beach seine net fishermen off the coast between Sunrise Point and Strandfontein Point in False Bay. *Rhinobatos annulatus* and *R. blochii* were collected from Saldanha Bay with the aid of a beach trek net (50m long and 1.5m deep) with a mesh size of 1 cm. All samples were collected intermittently between March 2013 and April 2014 (Table 4.1). There were 19 *Callorhinchus capensis*, 18 *Rhinobatos annulatus* and 13 *Rhinobatos blochii*.

Table 4.1: Collection details of samples of *Callorhinchus capensis* (CC), *Rhinobatos annulatus* (RA) and *Rhinobatos blochii* (RB) and the number of parasites utilized in the heavy metal analysis (*Gyrocotyle plana* and *Proleptus obtusus*).

Year	Date of capture	Host Species	Location	Host sample size (n)	Parasite species	Parasite sample size (n)
2013	March	RA	Saldanha Bay	3	*P. obtusus*	1
2013	May	CC	False Bay	9	*G. plana*	9
2013	June	CC	False Bay	2	*G. plana*	2
2013	June	RB	Saldanha Bay	13	*P. obtusus*	3
2013	November	CC	False Bay	8	*G. plana*	4
2013	November	RA	False Bay	15	*P. obtusus*	1

Samples were dissected as per the method in Chapters 2 and 3, which is outlined by MacKenzie and Abaunza, (2005). During parasitic data collection, samples of gonad, kidney, intestine (with bolus removed), liver and muscle were kept and re-frozen at -20^{o}C. Any macroscopic parasites were also kept and frozen. To prevent contamination, stainless steel dissection equipment was utilized between each dissection. All dissections were carried out on stainless steel counter tops. Frozen fish tissue samples were allowed to thaw at room temperature and 4g of sample were weighed off in acid washed glass Petri dishes, to the closest 0.001 gram (g). All glass wear was acid washed (2% hydrochloric acid bath) prior to weighing and use in ovens. The tissue samples were then dried for 48 hrs in a Memmert TV30 oven at 70°C. At 24 hrs the dried tissue samples were weighed and placed back into the ovens. Once 48hrs was completed, samples were re-weighed and these weights were compared to the 24 hrs measurement to determine if all moisture was removed. If the values were not consistent, then samples were put back in the oven for a further 24 hrs, or until the weight

measurements did not change. Once dried, the Samples were placed in plastic cryo.s™ (Greiner bio-one) vials and sealed with Parafilm M (plastic paraffin film) for transport to University of Johannesburg Zoology department and SPECTRUM Unit for digestion and metal analysis.

Once at the University of Johannesburg, samples were placed into a dissector (Sanpla Dry Keeper) till analysis. Samples were weighed to the closest 0.5 g with a Sartorius CP225D scale and placed into Teflon microwave digestion flasks (also referred to as "bombs"), along with 10 millilitres of 65% Suprapur™ Nitric acid (Merck, South Africa) and 1 millilitre of 30% Hydrogen peroxide. The digestion of the tissue samples was done using a CEM Mars 6 Microwave Reaction System for approximately two hours at 200°C. The samples were then diluted with Milli-Q water to 50ml and decanted into 50ml falcon tubes (Cellstar™ Tubes, greiner bio-one). The flacon tubes were then stored in a fridge at 4°C till analysis using an Inductively Coupled Plasma – Mass Spectrometer (Perkin Elmer, NexION 300 ICP-MS), located in the SPECTRUM Unit at the University of Johannesburg. Dog fish liver certified reference material for trace metals analysis was utilized for calibration and Gadolinium (Gd) was used as an internal standard.

4.2.2 Data Analysis

When surveys of the metal content of marine flora and fauna are conducted, a proportion of the samples may have metal concentrations that cannot be detected by the analytical method used (Ward et al., 1986). This will typically occur when a sample has a low concentration of the element and will often result in negative results. If these low values are ignored, an upward bias will occur, and if they are set to zero, this will both give a downward bias and lower the variance of the estimate. In this study, a method for determining the detection level set out by the International Union of Pure and Applied Chemistry (Thomsen et al., 2003) was used to take this bias into account:

$$X_L = X_{bi} + Ks_{bi}$$

Where X_L = Limit of detection, X_{bi} = Concentration of smallest measures (i.e.: Mean of blank measures), K = Numerical factor chosen according to the confidence level desired (for 90% confidence K = 3), s_{bi} = Standard deviation of blank measures.

These detection factors were calculated for each metal and any metal concentration measurement that was negative (i.e: undetectable) was replaced with the detection limit for that metal. Once detection limits were calculated and incorporated, data were converted to µg/g wet weight using

the moisture content of each individual tissue and their corresponding parasites. The following metals (with isotope number) and their associated detection limits are presented in Table 4.2. All these metals were analysed in tissue and parasite samples

Table 4.2: Metals tested for in *Callorhinchus capensis, Rhinobatos annulatus* and *R. blochii* and their parasites *Gyrocotyle plana* and *Proleptus obtusus* caught in False Bay and Saldanha Bay, South Africa between March 2013 and April 2014. The isotope number, symbol, measurement unit and the detection limits (X_L) for the ICP-MS are provided.

Analyte	**Symbol**	**Unit**	**Detection Limit**
Aluminium 27	Al	ppb	40.95
Arsenic 75	As	ppb	1.14
Cadmium 111	Cd	ppb	0.40
Cobalt 59	Co	ppb	1.55
Chromium 52	Cr	ppb	13.99
Copper 63	Cu	ppb	23.42
Manganese 55	Mn	ppb	0.15
Nickle 60	Ni	ppb	0.01
Lead 208	Pb	ppb	0.19
Antimony 121	Sb	ppb	1.67
Selenium 78	Se	ppb	3.49
Tin 118	Sn	ppb	1.41
Thorium 232	Th	ppb	1.01
Titanium 47	Ti	ppb	56.85
Uranium 232	U	ppb	0.20
Vanadium 51	V	ppb	0.09
Zinc 66	Zn	ppb	11.08

4.2.3 Statistical Analysis

The data were tested for normality and homogeneity of variances using Shapiro-Wilk test and Levene's tests respectively. Visual methods were utilized to confirm spread with frequency distributions and robust Quantile Quantile plots. Preliminary analyses of the data indicated a non-normal distribution, therefore non-parametric tests were incorporated.

To test if there was a species (*C. capensis*, *R. annulatus* and *R. blochii*) or location (False Bay and Saldanha Bay, only in *R. annulatus*) effect on metal accumulation, Kruskall Wallis and Wilcoxon non parametric rank tests were utilized. The metal concentration in the various tissues and their associated parasites are presented as mean concentrations (±standard deviation) and the significance between samples were tested using Kruskall Wallace signed rank test. Pairwise Wilcox tests were used as a post hoc to determine significance between tissue types and associated parasites. A Holm-Bonferroni correction (Holm, 1979) was used to counteract type I errors that arise with multiple comparisons.

All analyses were tested with a 95% confidence ($p<0.05$) and were conducted in either Microsoft Excel (2013) or R v 3.0.2 (R core Team, 2012). R packages used included Lawstat v 2.4.1 (Gastwirth et al., 2013) for robust normality tests such as Levene's Test of equality of variances and the production of QQ plots.

4.3 Results

4.3.1 Accumulation between species and location

Table 4.3 indicates a significant difference in total metal accumulation (averaged across all organs, excluding parasite) between species, with *R. annulatus* showing significantly more metal accumulation (Cr, Cu, Pb, Sb, Sn, Th, V, and Zn) than the other two species. *Rhinobatos blochii* accumulated the second most metals with *C. capensis* accumulating the lowest concentration of metals (As, Sb, Sn, and Zn) across the three species tested. Cd, Al, Co, Ni, and U were equally concentrated in all species.

Rhinobatos annulatus was the only species to be collected at both study sites. The Wilcox sign rank test investigated if there was a significant difference between the accumulation of metals within *R. annulatus* between the two locations (Table 4.4). The result indicated seven metal concentrations that were significantly different. Particularly, Co, Cr, Cu, Mn, Pb, Sn and Ti. Specimens from Saldanha Bay showed, on average, a higher accumulation of metals than specimens from False Bay.

4.3.2 Accumulation within tissues and parasites of specimens

Gyrocotyle plana was the only parasite to show significantly greater accumulation of As, Mn, Pb, Ti and Zn than its host's tissues and accumulated these metals to concentrations 2-6 times greater than the tissues of *C. capensis*. Not all metals indicated a significant difference between tissues of *C. capensis*, particularly Cr, Sb, and Sn. In *C. capensis*, the intestine and parasite accumulated the highest concentration of metals followed in order by gonad, kidney, liver and finally the muscle (Table 4.5).

In the two *Rhinobatos* species measured within this study, *R. annulatus* had significant differences in accumulation between all tissues except in Sb. The gonad of *R. annulatus* accumulated the highest concentration of metals of all tissues. Following the gonad is the intestine, muscle, liver, kidney and finally the host's parasite, *P. obtusus* (Table 4.6). *Rhinobatos blochii* also indicated significant differences in metal accumulation between organs with As, Sn, Th, and Ti being the exception. The intestine of *R. blochii* indicated the highest concentration of metals, followed by gonad, kidney, parasite, muscle, and finally the liver (Table 4.7).

Table 4.3: Mean concentrations (± standard deviation) of heavy metals present in *Callorhinchus capensis* (CC), *R. annulatus* (RA) and *R. blochii* (RB) caught between March 2013 and April 2014 in False Bay and Saldanha Bay. Significant differences demonstrated ($p<0.05$). Concentrations measured in µg/g.

Element	***C. capensis* (n=19)**		***R. annulatus* (n=18)**		***R. blochii* (n=13)**		**Significant Differences**
Al	476.15	(1868.63)	4476.39	(34995.01)	292.99	(474.66)	-
As	1590.66	(2572.73)	1794.11	(3188.11)	3403.80	(4016.43)	CC-RB, RA-RB
Cd	96.54	(337.34)	84.39	(194.35)	61.98	(125.67)	-
Co	34.23	(233.06)	12.92	(30.49)	7.37	(18.27)	-
Cr	40.22	(236.99)	96.73	(442.02)	19.73	(37.66)	CC-RA, RA-RB
Cu	546.37	(778.80)	598.14	(1502.87)	378.18	(663.22)	CC-RA, RA-RB
Mn	215.90	(376.78)	162.54	(648.22)	115.48	(214.63)	CC-RA
Ni	45.19	(257.81)	35.57	(94.14)	17.36	(50.37)	-
Pb	14.29	(42.47)	21.07	(92.75)	6.82	(16.53)	CC-RB
Sb	1.14	(4.91)	8.85	(19.62)	5.66	(15.04)	CC-RA, CC-RB
Se	330.69	(849.12)	265.44	(606.89)	315.60	(337.47)	CC-RA, RA-RB
Sn	0.98	(3.68)	7.52	(16.21)	4.27	(11.02)	CC-RA, RA-RB
Th	1.05	(5.57)	6.10	(41.02)	0.35	(0.46)	CC-RA, RA-RB
Ti	998.03	(1110.28)	321.92	(797.99)	824.65	(898.44)	CC-RA, RA-RB
U	0.59	(1.70)	2.58	(18.50)	0.24	(0.29)	-
V	13.78	(22.88)	25.17	(128.35)	7.45	(18.41)	CC-RA, CC-RB
Zn	4874.55	(7949.75)	15527.27	(56623.69)	8731.12	(16792.00)	CC-RA, RA-RB

Table 4.4: Mean concentrations (± standard deviation) of heavy metals present in *Rhinobatos annulatus* caught between March 2013 and April 2014 across the two localities (False Bay and Saldnha Bay) with significant differences demonstrated ($p<0.05$). Concentrations measured in µg/g.

Element	**False Bay (n=15)**		**Saldanha Bay (n=4)**		**Significant Differences**
Al	706.68	(2364.75)	11894.22	(60148.45)	-
As	2095.34	(3684.79)	1201.37	(1768.61)	-
Cd	105.10	(229.35)	43.63	(82.36)	-
Co	13.16	(25.95)	12.45	(38.38)	$p = 0.044$
Cr	59.37	(100.09)	170.23	(751.14)	$p = 0.022$
Cu	486.33	(1094.36)	818.16	(2092.96)	$p = 0.020$
Mn	103.97	(248.88)	277.79	(1063.00)	$p = 0.025$
Ni	35.25	(67.27)	36.20	(133.54)	-
Pb	10.83	(23.70)	41.22	(156.01)	$p < 0.001$
Sb	9.82	(19.87)	6.94	(19.30)	-
Se	280.48	(585.71)	235.83	(655.56)	-
Sn	7.38	(14.87)	7.81	(18.84)	$p = 0.007$
Th	2.06	(7.10)	14.04	(70.04)	-
Ti	82.16	(127.30)	793.70	(1245.92)	$p < 0.001$
U	0.46	(1.34)	6.75	(31.74)	-
V	13.76	(26.58)	47.62	(218.56)	-
Zn	21019.62	(68726.41)	4719.74	(9845.27)	-

Table 4.5: Mean concentrations and ± standard deviation of heavy metals present in the tissues of *Callorhinchus capensis* caught between March 2013 and April 2014 in False Bay. Significant differences between tissues demonstrated ($p<0.05$). Concentrations of the parasite, *Gyrocotyle plana* are also provided. Concentrations measured in µg/g.

Element	Gonad (n=19)	Intestine (n=19)	Kidney (n=19)	Liver (n=19)	Muscle (n=19)	*Gyrocotyle plana* (n=15)	Significant Differences
Al	56.61 34.30	2030.93 4207.59	261.67 460.50	222.49 286.38	57.09 51.04	161.96 166.02	-
As	1874.63 2109.44	732.80 757.97	985.24 921.09	1291.05 1327.62	1109.42 1289.79	4073.52 5561.54	I-P, K-P
Cd	244.52 781.48	41.08 49.01	92.15 98.57	142.93 155.82	10.46 39.51	35.16 33.03	M-P, L-M, K-M, G-M
Co	131.73 558.25	5.64 14.25	6.64 6.96	2.72 1.82	0.70 0.54	64.30 54.64	I-M, K-M, L-M, G-P, I-P, K-P, L-P, M-P
Cr	142.01 568.68	33.08 41.95	20.60 33.50	11.20 6.11	14.58 8.95	14.38 15.56	-
Cu	583.17 691.88	412.81 353.08	294.04 246.29	1370.98 1347.30	59.17 66.86	561.16 413.81	K-M, I-M, G-M, M-P
Mn	360.60 608.53	158.65 120.64	217.30 216.31	77.84 62.27	23.34 33.52	522.16 578.21	G-M, I-M
Ni	147.47 610.92	18.70 23.50	22.50 25.99	8.68 11.74	3.10 5.14	77.48 121.06	G-P, L-P, M-P, I-M
Pb	2.08 1.79	9.85 12.16	9.23 9.98	9.56 8.52	0.78 0.92	64.87 101.70	I-M, L-M,
Sb	0.74 1.28	3.14 11.71	0.66 0.56	0.86 0.69	0.57 0.68	0.78 1.02	-
Se	605.80 1395.65	139.89 188.02	759.75 1383.71	187.70 162.19	60.82 44.87	203.41 140.15	M-P
Sn	0.63 1.01	2.72 8.72	0.57 0.48	0.71 0.54	0.59 0.60	0.55 0.36	-
Th	0.73 1.48	4.28 13.11	0.28 0.51	0.27 0.42	0.33 0.48	0.24 0.34	I-K, I-L, I-M
Ti	1588.82 1634.09	687.47 609.38	672.09 639.46	325.37 286.80	1066.34 942.65	1821.42 1348.16	-
U	0.55 1.24	1.45 3.47	0.81 1.57	0.33 0.30	0.04 0.06	0.28 0.17	M-P, G-M, I-M, K-M, L-M
V	8.00 10.17	10.86 14.79	27.54 35.78	27.31 29.32	0.35 0.33	7.24 7.43	G-M, I-M, K-M, L-M
Zn	5816.85 7607.88	6493.51 12388.42	3582.62 3330.62	1320.31 1032.98	1187.88 2126.78	12438.57 9743.60	-

Table 4.6: Mean concentrations (± standard deviation) of heavy metals present in the tissues of *Rhinobatos annulatus* caught between March 2013 and April 2014 in False Bay. Significant differences between tissues demonstrated ($p<0.05$). Concentrations of the parasite, *Proleptus obtusus* are also provided. Concentrations measured in µg/g.

Element	Gonad (n=18)	Intestine (n=18)	Kidney (n=18)	Liver (n=18)	Muscle (n=18)	*Proleptus obtusus* (n=2)	Significant Differences
Al	362.64 869.56	22231.90 78355.69	36.09 12.20	116.49 115.24	77.02 70.62	496.99 493.06	G-I, I-K, I-L, I-M,
As	3470.47 4913.87	2402.99 3159.20	701.06 1132.91	246.09 497.22	2332.39 3309.67	152.13 181.72	G-M, I-M, K-M, L-M,
Cd	131.90 279.86	185.65 292.38	24.59 28.51	87.16 111.70	0.56 0.34	13.10 14.63	I-K, I-L
Co	34.87 35.23	27.35 50.53	1.38 0.41	1.29 1.00	0.98 0.67	1.36 0.27	G-K, I-K, G-M
Cr	96.88 131.36	329.80 974.30	12.43 3.63	12.66 3.91	40.35 52.07	20.25 27.74	G-M
Cu	791.80 1017.34	1819.32 2862.41	45.71 53.10	358.59 695.60	23.56 12.06	163.82 107.65	G-K, I-K, G-L, I-L, G-M, I-M
Mn	150.48 159.34	610.30 1393.86	20.09 40.60	34.93 33.87	7.03 12.44	71.29 69.27	I-L
Ni	51.52 67.26	86.62 176.91	21.52 73.34	2.42 3.74	19.40 37.70	2.82 2.68	G-I, I-K, I-L, I-M
Pb	27.54 29.25	68.28 203.60	8.17 17.38	1.26 1.58	2.09 4.44	3.18 1.57	G-K, I-K, I-L, G-M, I-M, L-M
Sb	27.65 28.16	14.10 25.79	1.31 0.70	1.38 0.70	0.77 0.83	0.09 0.08	G-K, G-L, I-L
Se	522.67 621.81	693.34 1071.90	32.98 57.94	57.88 72.27	38.21 43.04	104.45 135.46	I-K, I-K, I-L, G-M, I-M, L-M,
Sn	20.39 20.17	13.75 25.49	1.62 1.77	1.45 2.10	1.20 1.38	0.39 0.40	G-K, I-K, I-L, G-M, I-M, L-M
Th	0.85 0.37	28.30 91.42	0.69 0.63	0.55 0.82	0.75 0.44	0.29 0.41	G-L
Ti	229.35 303.07	812.88 1524.79	142.29 232.68	88.03 146.63	347.92 744.27	223.99 263.45	-
U	0.18 0.07	12.50 41.26	0.18 0.04	0.14 0.12	0.14 0.09	0.24 0.34	-
V	32.24 32.42	91.50 283.76	0.41 0.69	4.07 7.15	0.34 0.73	0.82 1.03	I-L
Zn	57816.22 115853.73	19376.61 33205.17	764.38 1302.08	1053.12 1418.67	169.48 322.83	1636.03 1132.08	G-K, I-K, K-L, G-M, I-M, L-M

Table 4.7: Mean concentrations (± standard deviation) of heavy metals present in the tissues of *Rhinobatos blochii* caught between March 2013 and April 2014 in False Bay. Significant differences between tissues demonstrated ($p<0.05$). Concentrations of the parasite, *Proleptus obtusus* are also provided. Concentrations measured in µg/g.

Element	Gonad (n=13)	Intestine (n=13)	Kidney (n=13)	Liver (n=13)	Muscle (n=13)	*Proleptus obtusus* (n=3)	Significant Differences
Al	167.81 178.73	626.05 731.62	154.13 159.83	373.35 624.50	63.94 58.44	638.10 347.44	I-M
As	4334.37 4687.57	2063.84 1864.88	3892.30 3649.39	1143.99 1265.14	6236.71 5503.65	577.68 513.77	-
Cd	42.40 60.73	69.17 109.17	128.64 239.42	74.86 71.02	0.70 0.49	36.52 55.64	G-M, I-M, K-M, L-M
Co	11.86 25.00	11.95 24.72	10.20 21.96	2.52 2.25	0.68 0.72	5.66 8.33	I-M
Cr	15.63 20.11	46.33 72.49	20.05 33.26	12.78 2.97	5.56 5.58	12.28 16.79	I-M, L-M
Cu	375.02 457.78	748.31 1020.80	202.10 170.44	523.99 902.01	30.16 15.33	427.24 383.66	G-M, I-M
Mn	75.27 65.44	221.73 380.58	218.43 249.11	43.89 25.88	11.33 9.53	144.73 116.88	-
Ni	35.37 92.54	18.69 30.31	30.49 59.65	2.80 2.95	0.89 0.89	11.20 16.07	-
Pb	6.80 14.13	10.75 22.99	16.74 24.06	0.69 1.18	0.48 0.35	1.03 1.44	K-L, K-M
Sb	9.16 20.25	9.07 18.99	8.84 20.10	1.77 0.73	0.57 0.76	0.71 0.66	L-M
Se	515.53 319.77	487.77 454.31	375.11 341.09	136.82 125.58	83.14 64.82	227.39 315.13	G-L, I-M,
Sn	6.47 14.50	6.76 15.38	7.01 13.50	1.44 1.31	0.60 0.67	0.27 0.24	-
Th	0.42 0.49	0.43 0.54	0.42 0.49	0.14 0.27	0.40 0.51	0.08 0.06	-
Ti	1048.34 875.57	930.19 624.21	707.33 598.00	210.99 152.21	837.67 663.44	2509.18 2825.82	-
U	0.15 0.07	0.55 0.52	0.16 0.05	0.24 0.19	0.09 0.09	0.19 0.10	G-I, I-K, I-M
V	9.99 22.78	11.92 26.09	13.08 22.89	3.61 4.56	0.19 0.23	0.89 0.54	I-M, L-M
Zn	17885.62 12475.03	18696.39 31672.92	5701.06 8120.54	1596.66 1274.63	368.28 305.53	6163.96 6483.48	G-L, I-L, G-M, I-M

4.4 Discussion

In this thesis, the accumulation of metals was studied in three shark species from two anthropogenically impacted bays in South Africa in order to identify a biological indicator of heavy metal accumulation. In previous chapters, two parasite species were identified as potential biological indicators, particularly *G. plana* that infects the intestine of *C. capensis* and *P. obtusus* that infects the stomach of *R. annulatus* and *R. blochii*. Within South African marine toxicology studies, there has been limited use of biological indicators, and only recently have results been released of the Mussel Watch Programme (Atkinson et al., 2006; Sparks et al., 2014). Previous work using biological indicators in South Africa was conducted approximately 30 years ago, therefore requiring an investigation into the potential for parasites and sharks as potential indicators. By conducting this work, this thesis can contribute to the current and limited literature we have on heavy metal accumulation in South Africa and hopefully provide new biological indicators to this important, yet, exclusive list.

By comparing the results above with baseline data obtained in Atkinson et al., (2006) and Mdzeke, (2004) (Table 4.7), the results above show that all three species of shark, *C. capensis*, *R. annulatus* and *R. blochii* are accumulating heavy metals within their tissues, confirming them as suitable candidates for biological indicator status. There is strong support for the use of higher trophic level animals as biological indicators, as they can bioaccummulate metals across a wider temporal and geographical scale than the surrounding water column and sediment (Dallinger et al., 1987). There is also considerable support for biomagnification of metals up the food chain, making higher trophic level organisms, such as sharks, more susceptible to accumulation than lower trophic level organisms (Al-Reasi et al., 2007; Sadiq, 1992).

These results also suggest a significant difference in the concentrations of heavy metals found between the different species of shark. This may be a reflection of species specific accumulation, storage and detoxifying strategies (Erasmus, 2004). However, various aspects such as diet, migration patterns, growth rates, trophic position, age structure of sampled animals, physiology, and environmental factors, or a combination of these, may affect metal concentrations in the various organs and tissues of these shark species (Erasmus, 2004). That *R. annulatus* and *R. blochii* are accumulating the highest concentrations, and that there were significant differences in most metals between the locations of *R. annulatus* suggests the rhinobatids residing in Saldanha Bay are accumulating higher concentrations of metals. To understand how their place of residence is

effecting this accumulation, it is worth noting the sources of heavy metals within these two locations and recording what research has been conducted and the concentrations that have been recorded.

Saldanha Bay is host to substantial commercial activities that subject the bay and its surrounds to various pollution inputs. These inputs are a direct result of a large tourism and fishing industry, seafood processing, steel works, iron-ore export facilities, domestic effluent and storm water runoff. Atkinson et al., (2006) released a State of the Bay report, which is a report that draws together all available information on water quality and aquatic health of Saldanha Bay and Langebaan Lagoon. Amongst other important factors, heavy metals were analysed in the sediment and a biological indicator organism was incorporated, either the Brown or Mediterranean mussel was used (*Perna perna* or *Mytilus galloprovicialis*). The report measured cadmium, copper, lead, zinc, iron and manganese concentrations and found the metals were well below the maximum legal limits prescribed for each contaminant in shellfish for human consumption, as stipulated by the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972). They concluded that there is no heavy metal accumulation in the flesh of mussels sampled in Small Bay of Saldanha Bay. However, measuring the trace metal concentrations in the sediment, they discovered that there has been substantial increase in concentration of metals, attributed to a substantial export of ore and an increase in dredging events. Cadmium concentrations recorded in 1999 far exceeded the safety threshold of 1.5 mg/kg established by the internationally accepted London Convention regulations. Fortunately safety thresholds set by the London Convention for Lead, Copper and Nickel were not exceeded, however, concentrations were considerably higher than had previously been recorded. The report further notes that from 2000 to 2004, metal concentrations have been decreasing and have attributed it to a burial of fine particle sediment and heavy metals by wave action.

False Bay is mainly under threat from domestic effluent and storm water runoff. There are a number of point source inputs that contribute to the pollution load that False Bay experiences. These include discharge points from sewage works and factory effluent around the bay. There is considerable runoff of herbicides and pesticides from intensive farming and major storm water runoff from urban areas. Shore and water based recreational activities with commercial fishing and bait collection are common uses of the beach and coastal areas. There is also substantial harbour activities from the Simon’s town naval base and Yacht club, Kalk bay fishing harbour and Gordon’s bay harbour. Mdzeke's, (2004) PhD dissertation looked at water, sediment and the accumulation of metals in shore based invertebrate species (*Oxystele tigrina, Oxystele sinensis, Choromytilus meridionalis, Patella oculus, Patiriella exigua* and *Tetraclita serrata*). She only measured cadmium, copper, nickel,

lead and zinc in her dissertation. Her results revealed that there was significant spatial and seasonal variation of metals along the False Bay coast with the invertebrates accumulating metals to concentrations higher than the surrounding water and sediment. She concluded that there has been an increase in heavy metal concentrations since the previous water quality surveys.

Both these studies (Atkinson et al., 2006; Mdzeke, 2004) have measured the sediment and invertebrates for accumulation of metals and found them to be affective accumulators of heavy metals and indicators of environmental pollution as a whole. However, comparing the concentrations of metals in *C. capensis* and *R. blochii* found in this study, to those found in the sediment and mussels of these two studies (Atkinson et al., 2006; Mdzeke, 2004), the sharks are accumulating some metals to orders of magnitude above that of the mussels (Table 4.8). Due to metal results in Atkinson et al., (2006) presented in figures, exact values were not available. Results from this thesis indicate how effective sharks are at accumulating heavy metals from the surrounding environment.

Table 4.8: Maximum element concentrations recorded in sediment and mussels from Mdzeke (2004) and Atkinson et al., (2006) with average metal values found in *C. capensis, R. annulatus* and *G. plana* found in this study. (–) indicate values not available

Element	**Sediment**		**Mussels**		**Sharks**		**Parasite**
	Mdzeke (2004) (µg/g)	**Atkinson (2006) (mg/kg)**	**Mdzeke (2004 (µg/g)**	**Atkinson (2006) (mg/L)**	***C. capensis* (µg/g)**	***R. blochii* (µg/g)**	***G. plana* (µg/g)**
Cd	12.36	6-7	16.2	>0.010	96.54	61.98	35.16
Cu	15.1	30-40	5	>0.010	546.37	378.18	561.16
Mn	-	-	-	>0.08	215.9	115.48	522.16
Ni	50	30	10.2	-	45.19	17.36	77.48
Pb	60.76	60-70	16.25	>0.25	14.29	6.82	64.87
Zn	119.55	-	273.5	>0.25	4874.55	8731.12	12483.57

What these summarized results also demonstrate is the stark difference between the two locations; Mdzeke, (2004) and *C. capensis* in False Bay and Atkinson et al., (2006) and *R. blochii* in Saldanha Bay. If we consider the sources of pollution mentioned above, with Saldanha Bay mainly impacted by industrial waste and False Bay by residential waste, and considering which metals the sharks are accumulating, there is chance for a distinction to be made. Mining and industrial effluent has been

shown to increase manganese, iron, chromium, zinc, lead and cadmium in the surrounding environment with domestic effluent and storm water runoff increasing arsenic, copper and cadmium. Unfortunately the shark results above and summarized data below do not indicate this trend conclusively, however there is some evidence for it in aluminium, cadmium, chromium and zinc.

One of the main objectives in this study was to identify if there is a parasite that could be used as a potential indicator of heavy metal accumulation. The parasite, *Gyrocotyle plana* was identified in Chapter 2 as a potential indicator for environmental change. The results above statistically show that *G. plana* is an incredibly good accumulator of certain metals, particularly As, Mn, Pb, Ti, and Zn. Table 4.7 shows that it does accumulate some of these metals to concentration orders of magnitude higher than the sediment and bivalves. Unfortunately *Proleptus obtusus* did not indicate itself as a potential indicator, however that could be attributed to its low sample size within the study (n=5) and that nematodes have been shown to be ineffective indicators of heavy metal accumulation (Otachi et al., 2014; Sures et al., 1999).

These results support work that has been done in other areas of the world, supporting intestinal helminths as exceptionally good accumulation indicators. Sures and Reimann, (2003) measured the acanthocephalan *Aspersentis megarhynchus* that infects the fish *Notothenia coriiceps* and found the parasite with metal concentrations orders of magnitude higher than the tissues of the host. Interestingly they found that the levels of metals in the tissue of the host was below the detection limit, however, the parasite accumulated the metals much more strongly. They concluded that if only the fish was measured, then one would report that the metals were in low enough concentration not to be bioavailiable for uptake. The parasite, though, due to its enormous uptake potential and the fact that it cannot regulate pollutants within itself showed that the metals were available in high enough concentration for uptake by organisms.

Within these sharks, results indicate the metals are accumulating in the gonads. This could be explained by a metals affinity with certain proteins, particularly storage proteins such as the metallothionein type. These metallothioneins occur in the liver, kidney and gills of fish with the purpose of regulating concentrations of essential metals in the metabolism. Unfortunately most metals that enter the system have high affinities for these compounds, whether essential to the fish's health or not (Bryan, 1971). That the kidney and liver have the ability to produce metallothioneins allows them to regulate the relative concentrations of metals within the organ and

as a proxy, the body. These organs also have the ability to excrete unwanted metals and transport essential metals to areas of use. This regulation could explain why the gonad, which is a protein rich storage organ (similar to the kidney and liver) has such a higher concentration, as it is not able to regulate the metal concentrations (Dallinger et al., 1987). Metallothioneins could also explain why the intestine is accumulating such high concentrations of metals compared to other tissues. Sures and Siddall (1999) pointed out that intestinal parasites, such as acanthocephalans and cestodes, are not able to synthesize their own cholesterol and fatty acids, they have become extremely efficient at taking them up from the intestinal lumen. Organometallic complexes (or metallothioneins) in the host bile pass down the bile duct into the small intestine where they are taken up by acanthocephalans concurrently with bile salts. This does explain why the parasites are absorbing the metals and could explain the presence of high concentrations of metals in the intestine.

4.4.1 Conclusions

There is, however, an incredible amount of questions still to be answered in this field, from expanding studies to monitoring a wider variety of pollution (e.g.: pesticides and hydrocarbons, to name a few), to including more species on the lists of potential indicators to also increasing sample area. A more directed effort is required, for example, the monitoring of harbours and bays where pollution is known to occur. Recommendations would include establishing a suite of indicators from different locations and from different trophic levels, to establish results for bioaccumulation, but more importantly, to establish temporal variation of metal accumulation by measuring biomagnification up the food web. Future studies should also include edibility calculations to establish whether organisms collected in an environment is safe for human consumption. Limitations experienced in this study include the limited sample sizes, which resulted in a large amount of variability. The limited abundance of *Proleptus obtusus* hindered the statistical analyses from drawing effective conclusions.

In this chapter, parasites and their shark hosts have demonstrated their incredible capability of accumulating heavy metals into their tissues, which supports the need for more effective long term monitoring into our marine ecosystems. By establishing long term monitoring programmes, we can begin to establish trends in marine pollution and understand the impact of anthropogenic activity on these organisms and the environment as a whole. Where the Mussel Watch Programme failed is the limited exposure the results have had onto the educated and general public. These results need to be released on a semi-annual basis so that policy can adapt to the changing pollution trends within

our ecosystems. If the results are not released, policy and management cannot adapt quick enough to prevent catastrophe from impacting the incredible diversity that South Africa has to offer.

4.5 References

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